



# ACTA OBSTETRICA ET GYNECOLOGICA SCANDINAVICA

## EDITORS

AXEL INGELMAN SUNDBERG DYRE TROLLE FINN BISE PAAVO VARA  
STOCKHOLM KÖBENHAVN OSLO HELSINKI

EDITOR IN CHIEF  
ALF SJÖVALL

## COLLABORANTES

E. BLOCK (Helsingfors) E. BRANDSTRAUP (Köbenhavn) S. V. FELDING  
(Köbenhavn) M. FURUKAWA (Stockholm) A. INGELMAN-SUNDBERG  
(Stockholm) E. JERLOW (Västbo) H. JONSSON (Stockholm)  
V. KAMANDER (Helsingfors) S. KUILLAND-MONDER (Dybbøl)  
H.-L. KOTTMEIER (Stockholm) P. KROGH (Odense) B. LUNDQVIST  
(Stockholm) L. LÖNNER (Bergen) I. NÄSLUND (Uppsala) C.  
NORSTRÖM (Helsingfors) A. OLSEN (Århus) ERIK PETTERSEN  
(Köbenhavn) Y. PLUM-FORSTELL (Kristiansund) L. SIMON (Norr-  
köping) S. SJÖSTRÖM (Linköping) S. THORSEN (Fakse) A. TULINEN  
(Helsingfors) P. VILJA (Helsingfors) E. WENSTEN (Kerstad) P.  
WETTERDAHL (Stockholm) H. ZILLIACUS (Helsingfors)

Vol XLVIII



# TABLE OF CONTENTS

## VOL XLVIII

### A

- Alexander Erik and Wägermark, Jan* Leydig cell tumours of the ovary  
Report of three cases 433
- Arumugam L. G.* History of contraception and introducing of intra-  
uterine contraceptive devices in rural area - Bandaragama, Ceylon 550
- Aukland Knut* see *Kibgenberg, Inge*
- Aure J Chr* see *Gjønnes H*
- Aure J Chr and Gjønnes H* Treatment of candidal vaginitis with  
Nifuratel 95
- Aure J Chr and Gjønnes H* Metronidazole treatment of trichomona-  
l vaginitis. A comparison of cure rates in 1961 and 1967 440

### B

- Bergström, J* see *Gellö M G*
- Bjerre Beate* Studies on population screening for early carcinoma of the  
cervix Suppl 6
- Bock Johannes E* The influence of prophylactic external cephalic ver-  
sion on the incidence of breech delivery. A retrospective study 215
- Bock Johannes E* Osteogenesis imperfecta. A report of case of the  
congenital form 222
- Brekke John and Westgren Anders* Liver reaction in connection with  
oral contraceptive steroids 242
- Bæ Fim* Studies on the human placenta. III. Vascularization of the  
young foetal placenta. A. Vascularization of the chorionic villus 159
- Bæ Fim* Studies on the human placenta. III. Vascularization of the  
young foetal placenta. B. Vascularization of the translocent villus and  
the syncytial band, with special reference to the cell islands and the  
basal plate 167

### C

- Castrén O, Laakso L and Nikkari T* The <sup>131</sup>I-triiodothyronine-rem-  
uptake test during labour 77





## J

- Jansson Ige see Spetz Sven
- Jansson I ge Iodine clearance in the myometrium of pregnant and non-pregnant women 302
- Jansson Ige Forearm and myometrial blood flow in diabetic pregnancy studied by venous occlusion plethysmography and Iodine clearance 322
- Jansson, Ige Peripheral and myometrial circulation in pregnancy Studies in normal toxæmic and diabetic pregnancies with venous occlusion plethysmography and Iodine tissue clearance Suppl. 8 p 5
- Jansson I ge Forearm and myometrial blood flow in toxæmia of pregnancy studied by venous occlusion plethysmography and Iodine clearance Suppl. 8 p 35
- Jergensen, P. I. Paracervical block Use as curettage for diagnostic purposes and for abortion 446

## K

- Kastrén, Tapio Gynecological health screening by means of questionnaire and cytology Suppl. 4
- Kidderén J. E. and Fjellström D. Cyclical variations in the arborescent crystal pattern of air-dried seminal fluid 147
- Kjängberg, Ige and Askblad Kent Measurement of human uterine cervical blood flow by local hydrogen gas clearance 455
- Kjängberg, Ige Measurement of blood flow in human myometrium by local hydrogen clearance 470
- Knoch, M. Herschlehl H. and Larsson J. Falck Ultrastructure of human choriocarcinoma 100
- Kokkonen Jukka see Koskela Osmo
- Koskela Osmo Kokkonen Jukka and Vahala Jaakko Resonographic studies of uterine patency following total hysterectomy 567
- Kristoffersen K., The significance of absence of one umbilical artery 195
- Kullander Stig and Wähl Lenaert The uterine cervix before and after cold-lead coarctation 258

## L

- Laakso L. see Casari O
- Laakso L. and Paasio J. Gastrointestinal protein loss in toxæmic patients 357
- Larsson J. Falck see Knoch M
- Larsson-Cohen Ulf The length of the first three menstrual cycles after combined oral contraceptive treatment 416
- Lundholm H. and Lundström P. Endogenous carbon monoxide production and blood loss at delivery 362
- Lundgren L. and Holmlund D. Friction between the foetal head and the cervix during labour 479

## F

- Felding, Carl F* Obstetric aspects in women with histories of renal disease Suppl. 2
- Fernst öm Ingemar Melin Karl-Gustav and Wikqvist Nils* Percutaneous catheterization of the hypogastric artery in local infusion therapy with cytostatics 119
- Fjellström D* see *Kihlström J E*
- Fjällbrant Bo* Sperm agglutinins in male blood donors 64
- Fjällbrant Bo* Cervical mucus penetration by human spermatozoa treated with anti-spermatozoal antibodies from rabbit and man 71
- Fjällbrant Bo* Studies on sera from men with sperm antibodies 131

## G

- Gelli M G Bergström J Hultman E, and Thalmé B* Heart muscle and plasma electrolytes in normal and glucose-loaded rabbit foetuses under anoxia 34
- Gelli M G and Gvulad F* Effect of glucose infusion in the mother before delivery on the ECG of rabbit foetuses under anoxia 56
- Gemzell Carl* Treatment of female and male sterility with human gonadotropins Suppl. 1 p 17
- Gjonnæss H and Aure J Chr* Treatment of trichomonas vaginitis with Nifuratel 85
- Gjonnæss H* see *Aure J Chr*
- Gvulad F* see *Gelli M G*

## H

- Hansson R Trydi g, N and Törnqvist A* Diamine oxidase (histaminase) in human pregnancy The activity of the enzyme in lymph and in blood plasma and the effect of heparin on the latter 8
- Hansson Roy* Diamine oxidase (histaminase) in blister fluid and blood plasma during pregnancy 19
- Hesseldahl H* see *Knorr M*
- Hibbard Bryan M and Hibbard Elizabeth D* The prophylaxis of folate deficiency in pregnancy 339
- Hibbard Bryan M and Hibbard Elizabeth D* The treatment of folate deficiency in pregnancy 349
- Hibbard Elizabeth D* see *Hibbard Bryan M*
- Holmlund D* see *Lidgren L*
- Hultman E* see *Gelli M G*

## I

- Ingólfsson Arni* Brow presentations 486



- Louwerens B* The clinical significance of the FSH/LH ratio in gonadotropin preparations of human origin. A review *Suppl 1* p 31
- Lundström P.*, see *Linderholm H*
- Lunell N-O., Persson B. and Sörkv G* Dietary habits during pregnancy. A pilot study 187
- Lundrall F. and Strakemann G* The urinary excretion of oestriol in pregnancies with Rh-immunisation 497

## M

- Melin Karl-Gustav* see *Fernström I gmar*

## N

- Nielsen Niels Chr* Coagulation and fibrinolysis in normal women immediately post partum and in newborn infants. Influence of prophylactic vitamin K 371
- Nielsen Niels Chr* Coagulation and fibrinolysis in diabetic women immediately post partum and in their newborn infants. Influence of Caesarean section 397
- Nielsen Niels Chr* Coagulation and fibrinolysis in women delivered by elective Caesarean section and in their newborn infants 405
- Nielsen Niels Chr* Coagulation and fibrinolysis in prematurely delivered mothers and their premature infants 505
- Nielsen Niels Chr* Influence of pre-eclampsia upon coagulation and fibrinolysis in women and their newborn infants immediately after delivery 523
- Nieminen Usko von Numers Claës and Purola Esko* Primary sarcoma of the ovary 423
- Nikkari T.* see *Castro O*
- von Numers Claës* see *Nieminen, Usko*

## O

- Official transactions* of the fifteenth meeting of the Northern Association of Obstetrics and Gynaecology held in Stockholm, June 6-8, 1968 *Suppl. 3*
- Ostergaard E.* see *O*

## P

- Paasio J.* see *Laakso L*
- Persson B.* see *Lunell N-O*
- Pierild Karl* Hysterography in the diagnosis of uterine myoma. Roentgen findings in 829 cases compared with the operative findings *Suppl 5*
- Proceedings* of the Symposium held under the auspices of Ercopharm A/S in Copenhagen, Sept. 1968. Hormonal treatment of infertility and oral anticonception *Suppl. 1*
- Purola Esko* see *Nieminen Usko*

## R

Rahn Kurt see Salonen L, Juhani

## S

- Salonen L, Juhani and Rahn Kurt Acute intermittent porphyria and pregnancy 1
- Saxén Lennart, see Tötterman L, Erik
- Sjöstedt Sven Induction of labour. A comparison of intranasal and trans-buccal administration of oxytocin Suppl. 7
- Sjöström Egid, Cancer of the female urethra. A clinical study of 25 cases 589
- Sjöström Jukka Sofie, Stress incontinence. A follow-up study of operative treatment 575
- Sjöström Sven and Jansson Ige Forearm blood flow during normal pregnancy studied by venous occlusion plethysmography and 133xenon muscle clearance 285
- Söderström G see Lundvall F
- Sterky G. see Lundvall, N-O
- Söderberg, Göran and Westin Björn Pressures at rest and during coughing in the bladder the uterus and the vagina at the onset of labour. A methodological study Suppl. 9

## T

- Tamule M The mechanism of action of oral contraceptives .Suppl. 1 p 41
- Thabae B see Gellä M G
- Tryding, N see Hansson R.
- Törnqvist A see Hansson, R.
- Törnqvist Åke The effect of histamine inhibition on the concentration and distribution of <sup>14</sup>C histamine in blood during pregnancy 272
- Tötterman L Erik and Saxén Lennart Incorporation of tetracycline into human foetal bones after maternal drug administration 542

## U

- Ulferts Magnus Kine Torsion of the pregnant human uterus 267

## V

- Vahala Jaakko see Koskela Osmo

## W

- Weklin Lennart see Kallander Stig
- Wenggren Anders see Brodhub Johan

- Louwerens B* The clinical significance of the FSH/LH ratio in gonadotropin preparations of human origin. A review *Suppl 1 p 31*
- Lundström P* see *Linderholm H*
- Lunell N-O Persson B* and *Sterky G* Dietary habits during pregnancy. A pilot study 187
- Lundvall F* and *Stakemann G* The urinary excretion of oestriol in pregnancies with Rh-immunisation 497

## M

- Melin Karl-Gustav* see *Fernström Ingmar*

## N

- Nielsen Niels Chr* Coagulation and fibrinolysis in normal women immediately post partum and in newborn infants. Influence of prophylactic vitamin K 371
- Nielsen Niels Chr* Coagulation and fibrinolysis in diabetic women immediately post partum and in their newborn infants. Influence of Caesarean section 397
- Nielsen Niels Chr* Coagulation and fibrinolysis in women delivered by elective Caesarean section and in their newborn infants 405
- Nielsen Niels Chr* Coagulation and fibrinolysis in prematurely delivered mothers and their premature infants 405
- Nielsen Niels Chr* Influence of pre-eclampsia upon coagulation and fibrinolysis in women and their newborn infants immediately after delivery 523
- Nieminen Usko von Numers Claës* and *Purola Esko* Primary sarcoma of the ovary 423
- Nikkari T* see *Castrén O*
- von Numers Claës* see *Nieminen, Usko*

## O

- Official transactions of the fifteenth meeting of the Northern Association of Obstetrics and Gynaecology* held in Stockholm, June 6-8, 1968 *Suppl. 3*
- Ostergaard E* see *O*

## P

- Paasto J* see *Laakso L*
- Persson B* see *Lunell N-O*
- Pietilä Karl* Hysterography in the diagnosis of uterine myoma. Roentgen findings in 829 cases compared with the operative findings. *Suppl. 5*
- Proceedings of the Symposium* held under the auspices of Ertopharm A/S in Copenhagen, Sept. 1968. Hormonal treatment of infertility and oral anticonception *S ppl 1*
- Purola Esko* see *Nieminen Usko*

*From the Department of Medicine (Professor W. J. Kaipainen) and the Department of Obstetrics and Gynaecology (Professor Pentti A. Järvinen) University of Oulu, Oulu, Finland*

## ACUTE INTERMITTENT PORPHYRIA AND PREGNANCY

BY

JUHANI SALOKANNEL AND KURT RHEN

Porphyria usually refers to a hereditary disorder of porphyrin metabolism, characterized by increased secretion of porphyrins in the urine. Large amounts of porphyrin metabolites are also found in the urine in some diseases and intoxications, e.g. in severe liver diseases, anaemia, cardiac infarction and heavy-metal intoxications (Watson 1963, Kiss and Szemere 1965, Schmid 1967).

At least fifteen forms of porphyria are recognized today. On the basis of clinical and biochemical differences, the porphyrias may be divided into two major groups: erythropoietic porphyria, in which the abnormal production of uroporphyrins occurs in normoblasts of the bone marrow, and hepatic porphyria, in which excessive amounts of porphobilinogen and uroporphyrins are synthesized in the liver. The most common is acute intermittent porphyria (AIP), which belongs to the hepatic group (Watson 1963, Herber 1965). Waldenström (1957) reported an incidence of 1.5 cases of AIP per 100,000 inhabitants in Sweden. The incidence in Finland has been presumed to be the same as in Sweden (Koskela 1962).

The onset of acute intermittent porphyria is usually heralded by abdominal colic, although neurological symptoms, such as weakened tendon reflexes and pareses of the peripheral and autonomic cerebral nerves, are common. In some patients the psychiatric symptoms, such as hallucinations and psychoses, predominate.



*Westin Björn see Söderberg, Göran*  
*Wigclist Nils see Fernström Ingmar*  
*Widgermark Jan see Allander Erik*

O

*Öckerman P. A.* Glucose-6-phosphatase in human endometrium 229  
*Ostergaard E.* Oral anticonception side effects and risks *Suppl. 1* p 57

## ACUTE INTERMITTENT PORPHYRIA AND PREGNANCY

BY

JUHANI SALOKANNEL AND KURT RHEN

Porphyria usually refers to a hereditary disorder of porphyrin metabolism, characterized by increased secretion of porphyrins in the urine. Large amounts of porphyrin metabolites are also found in the urine in some diseases and intoxications e.g. in severe liver diseases, anaemia, cardiac infarction and heavy-metal intoxications (Watson 1963, Riss and Szemere 1965, Schmid 1967).

At least fifteen forms of porphyria are recognized today. On the basis of clinical and biochemical differences the porphyrias may be divided into two major groups: erythropoietic porphyria, in which the abnormal production of uroporphyrins occurs in normoblasts of the bone marrow, and hepatic porphyria, in which excessive amounts of porphobilinogen and uroporphyrins are synthesized in the liver. The most common is acute intermittent porphyria (AIP), which belongs to hepatic group (Watson 1963, Harber 1965). Waldenström (1957) reported an incidence of 1.5 cases of AIP per 100,000 inhabitants in Sweden. The incidence in Finland has been presumed to be the same as in Sweden (Koskela 1962).

The onset of acute intermittent porphyria is usually heralded by abdominal colic, although neurological symptoms, such as weakened tendon reflexes, and pareses of the peripheral and autonomic cerebral nerves, are common. In some patients the psychic symptoms, such as hallucinations and psychoses, predominate.

AIP is inherited, according to the Mendelian laws of inheritance dominantly autosomally (Waldenström 1937) The incidence in women is approximately 15 times that in men (Watson 1954 Waldenström 1957) The most common age of onset is between 20 and 30 years (Waldenström 1957) In other words the fertile age of women. For this reason occasionally a woman with porphyria is pregnant and the possible influence of pregnancy and parturition on the course of porphyria must be assessed

The Department of Medicine and Department of Obstetrics and Gynaecology of University of Oulu have treated several members of one family for acute intermittent porphyria. The purpose is here to describe the effect of pregnancy and child birth on their disease and to advance an opinion on the basis of the experience thus gained and on the findings reported in the literature concerning the role of pregnancy in AIP

### Case reports

The family immigrated from East Karelia. The father was born May 26 1907 and is still living. He has had pain in the back and lower limbs for over 15 years and been treated for a sciatic syndrome. Sometimes after urinating in snow he noticed that the urine was red. His macroscopically clear urine was examined for porphyrins for the first time in 1956 in connection with the present study: none were demonstrable and sediment was normal.

The mother of the family was born March 18 1906 and exhibited no symptoms nor signs suggestive of porphyria.

No porphyria could, therefore be demonstrated objectively in either of the parents although the father's aches and the occasional red colour of the urine justify a suspicion that he had latent AIP.

The family had 13 children. Three had died before they were 3 years of age. As far as is known, the causes of their deaths were infectious diseases. One daughter died at the age of 31 of acute intermittent porphyria. The three sons living were healthy and had shown no symptoms suggestive of porphyria. Of the six daughters living, AIP has been diagnosed to date in five.

The eldest child in the family a daughter born April 24 1929 had normal deliveries in 1951 and 1953. AIP was diagnosed in 1955 at the beginning of her third pregnancy with abdominal pain as the first symptom. Subsequently the pregnancy continued uneventfully and delivery as normal. About a month post partum the abdominal pain recurred and the patient's urine turned dark in colour. The pain subsided in a few days but the patient became psychotic and was treated at the Central Mental Hospital of Oulu for

nearly three weeks. A year after this delivery the patient again became pregnant and from the beginning of the pregnancy complained of pain on both sides of the body. In the third month of gestation the pregnancy was terminated because of increasing symptoms of AIP and the patient was sterilized. She recovered rapidly and is still living. She has not needed treatment in hospital for porphyria.

The next daughter was found to have AIP at the age of 30 in 1951. The first symptoms were abdominal pain and vomiting, and the urine turned dark in colour. She had two miscarriages in 1956 and 1959 and two deliveries in 1959 and 1960. In connection with the latter delivery she was given penicillin. No symptoms of porphyria were evident during the pregnancies and the deliveries were normal. The patient died about one year after the diagnosis of AIP with symptoms of ascending paralysis.

The third case of porphyria in the family was diagnosed in 1962 in a daughter aged 21 years at the time. The first symptoms again were abdominal pain and vomiting, but she also had back-ache. The two years following the diagnosis were almost symptom-free until in November 1964 the patient came to our hospital asking for therapeutic abortion. She complained of increasing back ache but the principal reason she gave to justify her request for abortion was that she did not want to have a child which might have the same disease as she had. She was still unmarried. The patient's general condition was good and no neurological symptoms of porphyria were demonstrable. She was found to be in the 5th or 6th month of gestation. The calculated date of confinement was March 25 1965. Abortion was not considered justifiable. The patient married soon afterwards, and her pregnancy had an uneventful course only on two occasions for a few days each, did she have pain in legs and abdomen. She was examined at the Department of Obstetrics on February 11/12 1965 and was found to be subjectively symptom-free. Physical examination gave no abnormal findings. The Ehrlich test revealed porphobilanogen in the urine. The secretion of coproporphyrin per 24 hours was 209  $\mu\text{g}$  and of uroporphyrin 20,800  $\mu\text{g}$ .

On April 12, 1965 the patient was admitted for postnatality. She was subjectively free from symptoms of porphyria and physical examination again gave no abnormal findings. Her haemoglobin was 10.8 g/100 ml and haematocrit 32 per cent, serum bilirubin 0.9 mg % and serum glutamic oxaloacetic transaminase 55 Wroblewsky units per ml. The Ehrlich test was strongly positive. Schlemmer and the sodium test were negative. Oestrogens were administered during the morning of April 13 and regular labour pains started the same evening. A normal delivery followed 36 hours later the child was a healthy boy birth weight 3580 g. The placenta weighed 600 g, and histological study revealed placental calcification. The patient was given no drugs during or after parturition. On the day before parturition the urinary secretion of uroporphyrin per 24 hours was 11,580  $\mu\text{g}$ , the day after parturition was 18,020  $\mu\text{g}$ , and 9 days post partum 9022  $\mu\text{g}$ . Porphobilanogen was not found in the urine on all these occasions. During confinement the patient showed no symptoms suggestive of porphyria. Not until a year post partum

after ingestion of a barbiturate-containing drug for an acute infection, did the patient experience strong abdominal pain which subsided with rest in bed and chlorpromazine

The fourth case of porphyria in the family a girl was treated for mania at the Central Mental Hospital in Oulu at the age of 15. Then she was suspected to have porphyria but porphobilinogen in the urine could only be shown 4 years later in 1954 in connection with abdominal pain. In 1956 she contracted paraplegia, but recovered relatively well. She is unmarried and has no pregnancies. The youngest daughter was diagnosed as having porphyria recently at the age of 17.

In addition there is a daughter of 34 who has had two childbirths but no symptoms of porphyria and a daughter of 24 with a history of two childbirths and a miscarriage. In connection with the last delivery she was examined for porphyrins in the urine but none were found.

Acute intermittent porphyria has thus been diagnosed in five daughters of 13 children in the family. One of them died of the disease. Three of the five daughters with AIP have had a total of nine pregnancies of which six were carried to term, two ended with spontaneous abortion and one with induced abortion. Four deliveries occurred before the AIP had been diagnosed, in one case AIP was diagnosed at the beginning of pregnancy and in another case it had manifested itself two years before the pregnancy began. Both spontaneous abortions had occurred before the outbreak of the disease.

### *Discussion*

Exacerbations and remissions are common in acute intermittent porphyria. The severity of the attacks varies a great deal even in the same patient. This makes it difficult to assess the effect of pregnancy on its course. Reports on accumulated data in the literature usually indicate that pregnancy aggravates porphyria (Kiss and Szemere 1965, Seckler and Rovinsky 1965) while in occasional cases pregnancy has been associated with a remission (O'Dwyer 1955). The series collected by Seckler and Rovinsky (1965) consisted of 55 women with porphyria; they had 48 deliveries before and 67 deliveries after the recognition of the disease. In this series exacerbations occurred in three of four pregnancies while remission occurred only once in every six

pregnancies. In 24 cases the first attack occurred during pregnancy.

The porphyria patients of the family presented had six pregnancies before the outbreak of the disease. In one case AIP manifested itself during the third pregnancy. In this patient the disease was aggravated at the beginning of the next pregnancy which was terminated in the 16th week of gestation and the patient was sterilized. In one patient pregnancy did not affect the course of the porphyria diagnosed two years previously. One patient had four pregnancies, and AIP developed two years after the last pregnancy. It ended fatally after just over a year. On the basis of the present series pregnancy cannot be shown clearly to have provoked the onset of AIP or to have aggravated the disease. The death in the present series was unrelated to pregnancy whereas in Seckler's and Rovinsky's series (1965) the mortality rate during pregnancy was 22.4 per cent. On the other hand, it is difficult to attribute this mortality to pregnancy alone, since according to Waldenström (1937) the mortality rate within one year of the outbreak of AIP is 20 per cent.

On the basis of the series reported in the literature (Kiss and Szemere 1965, Seckler and Rovinsky 1965) it is apparent that exacerbation of porphyria is unusually frequent during pregnancy; some of these exacerbations are fatal. Notwithstanding this, according to Seckler and Rovinsky (1965) induced abortion is not indicated at least as long as the exacerbation is acute. Twenty three of the 67 pregnancies in their series ended with abortion and 11 patients died after the abortion; six of these abortions with fatal outcome were spontaneous and five were induced. The present series included one induced abortion after exacerbation of the symptoms of AIP. It was followed by remission. The present authors find that termination of pregnancy should be considered individually in each case, weighing the psychobiological factors which also may be responsible for the exacerbation of AIP (Harrison 1963).

There is no set rule concerning the method of delivery that should be chosen for patients with acute intermittent porphyria. Kiss and Szemere (1965) preferred Caesarean section to vaginal delivery since the moment of delivery can be chosen, the psychic

after ingestion of a barbiturate-containing drug for an acute infection, did the patient experience strong abdominal pain which subsided with rest in bed and chlorpromazine.

The fourth case of porphyria in the family a girl was treated for mania at the Central Mental Hospital in Oulu at the age of 15. Then she was suspected to have porphyria but porphobilinogen in the urine could only be shown 4 years later in 1954 in connection with abdominal pain. In 1956 she contracted paraplegia but recovered relatively well. She is unmarried and has no pregnancies. The youngest daughter was diagnosed as having porphyria recently at the age of 17.

In addition there is a daughter of 34 who has had two childbirths but no symptoms of porphyria, and a daughter of 24 with a history of two childbirths and a miscarriage. In connection with the last delivery she was examined for porphyrins in the urine but none were found.

Acute intermittent porphyria has thus been diagnosed in five daughters of 13 children in the family. One of them died of the disease. Three of the five daughters with AIP have had a total of nine pregnancies of which six were carried to term two ended with spontaneous abortion and one with induced abortion. Four deliveries occurred before the AIP had been diagnosed in one case AIP was diagnosed at the beginning of pregnancy and in another case it had manifested itself two years before the pregnancy began. Both spontaneous abortions had occurred before the outbreak of the disease.

### *Discussion*

Exacerbations and remissions are common in acute intermittent porphyria. The severity of the attacks varies a great deal even in the same patient. This makes it difficult to assess the effect of pregnancy on its course. Reports on accumulated data in the literature usually indicate that pregnancy aggravates porphyria (Kiss and Semere 1965, Seckler and Rovinsky 1965) while in occasional cases pregnancy has been associated with a remission (O'Dwyer 1955). The series collected by Seckler and Rovinsky (1965) consisted of 55 women with porphyria; they had 48 deliveries before and 67 deliveries after the recognition of the disease. In this series exacerbations occurred in three of four pregnancies while remission occurred only once in every six

pregnancies. In 24 cases the first attack occurred during pregnancy

The porphyria patients of the family presented had six pregnancies before the outbreak of the disease. In one case AIP manifested itself during the third pregnancy. In this patient the disease was aggravated at the beginning of the next pregnancy which was terminated in the 16th week of gestation and the patient was sterilized. In one patient pregnancy did not affect the course of the porphyria diagnosed two years previously. One patient had four pregnancies, and AIP developed two years after the last pregnancy. It ended fatally after just over a year. On the basis of the present series, pregnancy cannot be shown clearly to have provoked the onset of AIP or to have aggravated the disease. The death in the present series was unrelated to pregnancy whereas in *Seckler's* and *Rovinsky's* series (1965) the mortality rate during pregnancy was 22.4 per cent. On the other hand, it is difficult to attribute this mortality to pregnancy alone since according to *Waldenström* (1937) the mortality rate within one year of the outbreak of AIP is 20 per cent.

On the basis of the series reported in the literature (*Kiss and Szemere* 1965, *Seckler and Rovinsky* 1965) it is apparent that exacerbation of porphyria is unusually frequent during pregnancy; some of these exacerbations are fatal. Notwithstanding this, according to *Seckler and Rovinsky* (1965) induced abortion is not indicated at least as long as the exacerbation is acute. Twenty three of the 67 pregnancies in their series ended with abortion, and 11 patients died after the abortion; six of these abortions with fatal outcome were spontaneous and five were induced. The present series included one induced abortion after exacerbation of the symptoms of AIP. It was followed by remission. The present authors find that termination of pregnancy should be considered individually in each case, weighing the psychobiological factors which also may be responsible for the exacerbation. (AIP) (*Watson* 1963)

There is no set rule concerning the method of delivery that should be chosen for patients with acute intermittent porphyria. *Kiss and Szemere* (1965) preferred Caesarean section to vaginal delivery. Since the moment of delivery can be chosen, the psychic



excitement associated with delivery can be avoided, and the local anaesthetics of adverse effect, are not required. In the present series the deliveries were effected by the normal vaginal route, and no exacerbations occurred. The present authors find that operation is a bigger stress on patient than vaginal delivery and the anaesthetics used are an additional factor (*Seckler and Rovinsky 1965*)

Surgical sterilization of AIP patients is considered contra indicated but the patient should be advised to use an efficient contraceptive method (*Seckler and Rovinsky 1965 Zilliacus 1967*). Today an intrauterine device is probably the most suitable method since oral contraceptive pills are claimed possibly to provoke AIP attacks (*Schmid 1967*). But it has also been claimed that in certain cases oral contraceptives may have prevented AIP attacks (*Perroth et al 1965*)

In conclusion, it seems desirable that women with AIP should not become pregnant. But once pregnancy has started the patient's general condition should be kept as good as possible and drugs provoking attacks such as barbiturates sulphonamides alcohol and ergotamine should be avoided (*Seckler and Rovinsky 1965 Schmid 1967*). Surgical operations should be avoided during pregnancy unless necessitated by patient's obstetric condition. Chlorpromazine (*Schmid 1967*) is probably the most suitable drug to reduce the symptoms of porphyria. Also cortisone is found to exert a beneficial effect on porphyria during pregnancy (*Zilliacus 1967*). With adequate treatment, delivery can take place in the normal way without exacerbation of the symptoms of porphyria.

## SUMMARY

A family with acute intermittent porphyria (AIP) is presented and the possible effect of pregnancy and delivery on the clinical picture is described. The five daughters of the family affected with porphyria, have had a total of nine pregnancies of which six were carried to term, two ended with spontaneous abortion and one with induced abortion. Pregnancy and childbirth in the present series could not be clearly shown to provoke the onset or exacerbation of AIP during pregnancy.

## REFERENCES

- Harber L. C., *Med. Clin. N Amer.* 49: 581 1965  
Kiss Cs and Szemere P. *Zentralbl. Gynäk.* 32: 1117 1965  
Koskela E. *Duodecim* 78: 1105 1962  
O'Dwyer J. P. *J. Obst. & Gynec. Brit. Emp.* 62: 437 1955  
Perroth M. G., Merner H. S. and Tachydy D. P. *J.A.M.A.* 194: 1037 1963  
Schärdl, R. *Textbook of Medicine* Twelfth edition, Philadelphia and London 1957  
Seckler S. G. and Rostovsky J. J. *Medical Surgical and Gynecologic Complications of Pregnancy* Second edition Baltimore 1963  
Waldenström J. *Acta med. scand. Suppl.* 82: 1937  
*Ann. J. Med.* 22: 768 1957  
Watson C. J. *Advances Int. Med.* 6: 235 1954  
*Textbook of Medicine*, Eleventh edition Philadelphia and London 1963  
Zilberstein H. *Ann. Chir. Gynaec. Fenn.* 56: 349 1967

Received on Feb. 12, 1968

excitement associated with delivery can be avoided and the local anaesthetics of adverse effect are not required. In the present series the deliveries were effected by the normal vaginal route and no exacerbations occurred. The present authors find that operation is a bigger stress on patient than vaginal delivery and the anaesthetics used are an additional factor (Seckler and Rovinsky 1965).

Surgical sterilization of AIP patients is considered contra indicated, but the patient should be advised to use an efficient contraceptive method (Seckler and Rovinsky 1965, Zilliacus 1967). Today an intrauterine device is probably the most suitable method since oral contraceptive pills are claimed possibly to provoke AIP attacks (Schmid 1967). But it has also been claimed that in certain cases oral contraceptives may have prevented AIP attacks (Perrothi et al 1965).

In conclusion it seems desirable that women with AIP should not become pregnant. But once pregnancy has started, the patient's general condition should be kept as good as possible and drugs provoking attacks such as barbiturates, sulphonamides, alcohol and ergotamine should be avoided (Seckler and Rovinsky 1965, Schmid 1967). Surgical operations should be avoided during pregnancy unless necessitated by patient's obstetric condition. Chlorpromazine (Schmid 1967) is probably the most suitable drug to reduce the symptoms of porphyria. Also cortisone is found to exert a beneficial effect on porphyria during pregnancy (Zilliacus 1967). With adequate treatment, delivery can take place in the normal way without exacerbation of the symptoms of porphyria.

### SUMMARY

A family with acute intermittent porphyria (AIP) is presented and the possible effect of pregnancy and delivery on the clinical picture is described. The five daughters of the family affected with porphyria have had a total of nine pregnancies of which six were carried to term, two ended with spontaneous abortion, and one with induced abortion. Pregnancy and childbirth in the present series could not be clearly shown to provoke the onset or exacerbation of AIP during pregnancy.

## REFERENCES

- Harber L. C. *Med. Clin. N Amer* 49 581 1965  
Kiss Cs. and Szemere P. *Zentralbl. Gynäk.* 32 1117 1965  
Koskela P. *Duodecim* 78 1105 1962  
O'Donovan J. P. *J. Obst. & Gynec. Brit. Emp.* 62 437 1955  
Perroth M. G. Marner H. S. and Tschudy D. P. *J.A.M.A.* 194 1037 1965  
Schmidt R., *Textbook of Medicine* Twelfth edition Philadelphia and London 1977  
Seckler S. G. and Roubusky J. J. *Medical Surgical and Gynecologic Complications of Pregnancy* Second edition, Baltimore 1965  
Waldenström J. *Acta med. scand. Suppl.* 82 1937  
*Am. J. Med.* 22 766 1957  
Watson C. J. *Advances Int. Med.* 6 235 1964  
*Textbook of Medicine* Eleventh edition, Philadelphia and London 1963  
Zilliacus H. *Ann. Chir. Gynaec. Fenn* 56 349 1967

Received on Feb 12, 1968

excitement associated with delivery can be avoided and the local anaesthetics of adverse effect, are not required. In the present series the deliveries were effected by the normal vaginal route, and no exacerbations occurred. The present authors find that operation is a bigger stress on patient than vaginal delivery and the anaesthetics used are an additional factor (Seckler and Rovinsky 1965)

Surgical sterilization of AIP patients is considered contra indicated but the patient should be advised to use an efficient contraceptive method (Seckler and Rovinsky 1965 Zilliacus 1967). Today an intrauterine device is probably the most suitable method since oral contraceptive pills are claimed possibly to provoke AIP attacks (Schmid 1967). But it has also been claimed that in certain cases oral contraceptives may have prevented AIP attacks (Perroth *et al* 1965)

In conclusion it seems desirable that women with AIP should not become pregnant. But once pregnancy has started, the patient's general condition should be kept as good as possible and drugs provoking attacks such as barbiturates sulphonamides alcohol and ergotamine should be avoided (Seckler and Rovinsky 1965 Schmid 1967). Surgical operations should be avoided during pregnancy unless necessitated by patient's obstetric condition. Chlorpromazine (Schmid 1967) is probably the most suitable drug to reduce the symptoms of porphyria. Also cortisone is found to exert a beneficial effect on porphyria during pregnancy (Zilliacus 1967). With adequate treatment, delivery can take place in the normal way without exacerbation of the symptoms of porphyria.

### SUMMARY

A family with acute intermittent porphyria (AIP) is presented and the possible effect of pregnancy and delivery on the clinical picture is described. The five daughters of the family affected with porphyria, have had a total of nine pregnancies of which six were carried to term, two ended with spontaneous abortion, and one with induced abortion. Pregnancy and childbirth in the present series could not be clearly shown to provoke the onset or exacerbation of AIP during pregnancy.

Table I. Details of Cases

Subject		Age (Years)	Pregnancy		Cause of Stay in Hospital (H=Healthy Volunteers)
Group	No.		No.	Estimated Duration (Weeks)	
A.	1	29	II	37	Diffuse pulmonary changes rather military discovered at a mother care visit. No definite diagnosis. Scapular fat pad nodes showed nothing abnormal. Lung biopsy specimens revealed granulomatous changes suggestive of tuberculosis but no mycobacteria have ever been found in tissues sputa or gastric washings.
B	1	35	IV	41	Rh-immunization (insignificant)
	2	36	V	42	Social reason
	3	23	II	42	Rh-immunization (insignificant)
	4	30	III	31	H
	5	19	I	41	Suspected postmaturity increased weight
C	1	19	I	38	II
	2	28	I	27	H
	3	26	I	37	H
	4	25	I	32	H
	5	25	II	31	II
	6	24	II	33	H
	7	29	II	29	H
	8	19	I	34	H
	9	27	I	38	H

Case A. Venous blood and thoracic duct lymph sampling only

Cases B. Venous blood sampling before and (Fig. 1) after injection of 10,000 IU of Heparin® (Vitrum) within 15 seconds

Cases C. Capillary sampling only at (within  $\pm 10$  minutes) 8 a.m. noon 4 and 8 p.m.

significant amount of the DAO in the bloodstream in men and non-pregnant women after administration of heparin is transported there by the lymph. Assuming the conventional concept of the transport of lymph from the uterus, and a transport of DAO by the lymph, the DAO-activity of the thoracic duct lymph

## DIAMINE OXIDASE (HISTAMINASE) IN HUMAN PREGNANCY

The Activity of the Enzyme in Lymph and in Blood Plasma and the Effect of Heparin on the Latter

BY

R. HANSSON, N. TRYDING AND Å. TÖRNQVIST

The human placenta contains large amounts of diamine oxidase (DAO) (Danforth and Gorham 1937 Ahlmark 1944 Swanberg, 1950 Kapeller Adler 1965 Southren et al 1965 and 1966)

The increase of this enzyme in the blood during pregnancy (Marcou et al 1938) derives at least partly from the decidual cells of the placenta (Swanberg, 1950) It appears that no other sources of the DAO in human plasma during pregnancy have been reported in the literature

In human beings intravenous administration of heparin results in an increase of the DAO-activity of the blood plasma to levels otherwise seen only during the latter part of pregnancy (Tryding, 1965 a) This effect of heparin varies with the dose given and from one individual to another and appears to be ascribable to a release of DAO into the bloodstream and lymph (Hansson et al 1966 Dahlbäck et al. 1968)

The DAO-activity of human lymph is normally about 30 times higher than that of the blood and this relation will increase to more than 100 times after administration of 10 000 IU heparin, despite a simultaneous marked rise of the activity in the blood plasma (Dahlbäck et al 1968) It would thus appear that a

calculated from a series of  $n$  double determinations as  $\sqrt{\sum d^2 / 2n}$ , where  $d$  is the difference between  $n$  duplicate determinations)

*Calculation.* For each of the nine normal subjects in group C the average value on the day of investigation,  $\frac{\sum X}{4}$ , was estimated and taken as 100 %. The four results were expressed as percentages thereof. The 36 results showed a normal distribution, except for the noon value in patient 9 which was therefore excluded. For each of the nine subjects the standard deviations ( $= \sqrt{\sum \text{squares of percentage differences} / 3}$ ) and the coefficients of variation ( $= \frac{SD}{\text{mean}} \times 100$ ) were calculated. Finally the mean value of this coefficient, the standard deviation and standard error of the mean were estimated.

*Results.* The DAO-activity of thoracic duct lymph from woman A1 was 0.50 U/l. The corresponding value for the blood plasma was 1.53 U/l, i.e. 3 times as high.

The spontaneous diurnal variation in pregnant women is given in Table II. The variation coefficient for a 12-hour period ranged between 3 and 15 % with a mean of 5 % ( $\pm 2\%$  = S.D. A possibly discernible diurnal variation is receiving further attention for later publication).

*After heparin.* The initial level of the DAO-activity in blood plasma was normal for the stage of pregnancy. The administration of heparin produced no symptoms or signs of any side reactions.

Within 2 minutes of the injection of heparin the two women, who were examined at short intervals, showed a plasma-DAO activity which rose to three-fourths of the maximum recorded (Fig. 1). Maximum activity was achieved after approximately 20 minutes in both of the women. In the woman who was examined one hour after a meal the activity curve declined smoothly. For the pregnant woman who had abstained from food and water for more than 12 hours before the test the plasma DAO-activity curve clearly undulated for the first 90 minutes



should be higher than in non pregnant subjects and higher than in the blood plasma. For determining the DAO activity of duct lymph and comparing it with that of the blood plasma, lymph was aspirated from the thoracic duct (four weeks before parturition) in a pregnant woman undergoing left sided biopsy of the scalenus lymph node for diagnosis of pulmonary changes.

Five women in the last trimester of pregnancy were studied for the effect of pregnancy on the liberation of DAO after intravenous injection of heparin. The DAO activity of the blood plasma was determined before and repeatedly during the first few hours after intravenous injection of heparin. Since a search of the literature failed to reveal any investigation of the diurnal variation of any of the DAO-activity of the blood plasma during pregnancy the activity was followed for a 12 hour period in 9 healthy pregnant women.

### *Clinical Material and Methods*

The series comprised patients without any appreciable obstetric complications (see Table 1). Heparin® (Vitrum) 5000 IU/ml (cont. tricesol 2 mg/ml) was used. DAO. The diamine oxidase (DAO) activity was determined by Trydling's (1965 b) modification of Okuyama & Kobayashi's method. A mixture of 50  $\mu$ l of the  $^{14}$ C-putrescine 1 mM and 50  $\mu$ l of plasma or lymph is incubated at 37 °C in 1/15 M phosphate buffer pH 7.4. The reaction product, 1 pyrroline but not the putrescine which remains in the water phase is extracted quantitatively by ready prepared scintillator fluid and the  $^{14}$ C activity is counted in a liquid scintillation counter (Packard 2000-series). One unit is defined as the enzyme activity which under the prevailing conditions of incubation will convert 1  $\mu$ mole of the substrate per minute.

The reaction is of zero order for DAO activity up to 3 U/l if the enzyme concentration is higher, shorter incubation or a smaller amount of the sample is chosen.

Non-pregnancy values in blood plasma 0-25 mU/l are determined with a random error of approximately 20 % whereas normal values from the second or third trimester are determined with a random error of about 1 % (The error of the method was

# DAO in Blood Plasma in Pregnancy

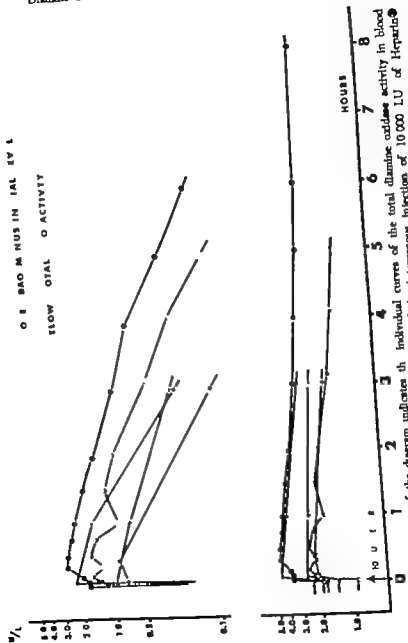


Fig. 1 The lower part of the diagram indicates the individual curves of the total diamine oxidase activity in blood plasma whereas the upper part presents the net effect of the intravenous injection of 10 000 LU of Heparin (Vitrum).

Table II Results

Subject	Group	No	DAO Values (U/l) in Blood Plasma (Unless Otherwise Stated)													
			Before Heparin	After Heparin (Minutes)												
			2	5	10	20	0	45	60	90	120	180	240	300	360	
1			Lymph. 0.50 blood plasma. 1.53													
1			1.39	2.06		2.23						1.47				
2			1.75	2.66					2.40			1.96				
3			2.17	4.57					3.80			2.54				
4			.93	2.07	2.23	2.44	2.11	2.46	2.27	1.75	1.98	1.75	1.30	1.14	1.03	-
5			1.92	3.59	3.60	3.82	4.53	4.51	4.21	4.04	3.61	3.19	2.69	2.44	2.14	2.04
1			.81													
2		.83	.87													
3		2.06	1.99													
4		1.18	1.15													
5		.88	.95													
6		1.21	1.00													
7		2.96	2.79													
8		1.58	1.58													
9		2.14	1.84													
10		(.34)	.58													
11		noon	4 p.m.													
12		8 a.m.	8 p.m.													
13		8 a.m.	8 p.m.													
14		8 a.m.	8 p.m.													
15		8 a.m.	8 p.m.													
16		8 a.m.	8 p.m.													
17		8 a.m.	8 p.m.													
18		8 a.m.	8 p.m.													
19		8 a.m.	8 p.m.													
20		8 a.m.	8 p.m.													
21		8 a.m.	8 p.m.													
22		8 a.m.	8 p.m.													
23		8 a.m.	8 p.m.													
24		8 a.m.	8 p.m.													
25		8 a.m.	8 p.m.													
26		8 a.m.	8 p.m.													
27		8 a.m.	8 p.m.													
28		8 a.m.	8 p.m.													
29		8 a.m.	8 p.m.													
30		8 a.m.	8 p.m.													
31		8 a.m.	8 p.m.													
32		8 a.m.	8 p.m.													
33		8 a.m.	8 p.m.													
34		8 a.m.	8 p.m.													
35		8 a.m.	8 p.m.													
36		8 a.m.	8 p.m.													
37		8 a.m.	8 p.m.													
38		8 a.m.	8 p.m.													
39		8 a.m.	8 p.m.													
40		8 a.m.	8 p.m.													
41		8 a.m.	8 p.m.													
42		8 a.m.	8 p.m.													
43		8 a.m.	8 p.m.													
44		8 a.m.	8 p.m.													
45		8 a.m.	8 p.m.													
46		8 a.m.	8 p.m.													
47		8 a.m.	8 p.m.													
48		8 a.m.	8 p.m.													
49		8 a.m.	8 p.m.													
50		8 a.m.	8 p.m.													
51		8 a.m.	8 p.m.													
52		8 a.m.	8 p.m.													
53		8 a.m.	8 p.m.													
54		8 a.m.	8 p.m.													
55		8 a.m.	8 p.m.													
56		8 a.m.	8 p.m.													
57		8 a.m.	8 p.m.													
58		8 a.m.	8 p.m.													
59		8 a.m.	8 p.m.													
60		8 a.m.	8 p.m.													
61		8 a.m.	8 p.m.													
62		8 a.m.	8 p.m.													
63		8 a.m.	8 p.m.													
64		8 a.m.	8 p.m.													
65		8 a.m.	8 p.m.													
66		8 a.m.	8 p.m.													
67		8 a.m.	8 p.m.													
68		8 a.m.	8 p.m.													
69		8 a.m.	8 p.m.													
70		8 a.m.	8 p.m.													
71		8 a.m.	8 p.m.													
72		8 a.m.	8 p.m.													
73		8 a.m.	8 p.m.													
74		8 a.m.	8 p.m.													
75		8 a.m.	8 p.m.													
76		8 a.m.	8 p.m.													
77		8 a.m.	8 p.m.													
78		8 a.m.	8 p.m.													
79		8 a.m.	8 p.m.													
80		8 a.m.	8 p.m.													
81		8 a.m.	8 p.m.													
82		8 a.m.	8 p.m.													
83		8 a.m.	8 p.m.													
84		8 a.m.	8 p.m.													
85		8 a.m.	8 p.m.													
86		8 a.m.	8 p.m.													
87		8 a.m.	8 p.m.													
88		8 a.m.	8 p.m.													
89		8 a.m.	8 p.m.													
90		8 a.m.	8 p.m.													
91		8 a.m.	8 p.m.													
92		8 a.m.	8 p.m.													
93		8 a.m.	8 p.m.													
94		8 a.m.	8 p.m.													
95		8 a.m.	8 p.m.													
96		8 a.m.	8 p.m.													
97		8 a.m.	8 p.m.													
98		8 a.m.	8 p.m.													
99		8 a.m.	8 p.m.													
100		8 a.m.	8 p.m.													

Comments on group C The noon value in subject C9 is not included. If the individual mean value be taken as=100% the mean value at 8 a.m. is 102% (S.D.=5.7%)  
 noon=104% (S.D. 5.1%)  
 4 p.m.=98% (S.D. 4.2%)  
 8 p.m.=96% (S.D. 2.9%)  
 Mean value at 8 p.m. < Mean value at 8 a.m. and noon (0.01 > p > 0.005)  
 If any difference is considered fiducial the percentage values can be accepted as a normal distribution with S.D.=5.4%

in men and in non-pregnant women of the same age groups (Hansson *et al.* to be published). This applies particularly to the time of maximum activity which in the 2 women studied most thoroughly occurred about 20 minutes after the injection, compared with 60-120 minutes in men and non-pregnant women. A similar tendency to an early maximum increase of the enzyme was noted also in the other 3 pregnant women, a finding rarely seen in non-pregnant individuals. The heparin-induced increase of the DAO-activity in the blood plasma in the pregnant women did not differ significantly in magnitude from that in non-pregnant women (Hansson *et al.* to be published) but was comparatively large. The slope of the DAO-curve was similar for all the pregnant women, and in the 2 women who were followed longest in the group the curves for enzyme activity approached the original level smoothly.

The increase in the DAO-activity of the blood plasma disappeared with a half time of 70-80 minutes in 2 and at approximately the same rate in the other women in the group. Also in this respect the pregnant women resembled the non-pregnant ones in whom the half time varied between 45 and 90 minutes (Hansson *et al.* to be published).

These half time values differ considerably from the post partum blood plasma findings (Ahlmärk 1944). Even if the pregnancy initiated histaminase pool that remains in the body after the delivery of the placenta, will retard the decrease in plasma histaminase the difference found III between a half-time of about 75 minutes in the post-heparin group and a half time of 25 to 30 hours in the post-partum group. This difference would presumably have been greater but for the prolonged lymph infusion, rich in DAO (histaminase) in the post-heparin group (Hansson *et al.* 1966 Dahlbäck *et al.* 1968). If the post-heparin DAO enzyme molecule is identical with the pregnancy-DAO in blood plasma the different eliminatory rates are an interesting finding. Pregnancy does not markedly influence the elimination of post heparin DAO from plasma. The possibility that there is a puerperal retardation of the elimination of any DAO-activity cannot be ruled out, but the existence of isoenzymes of DAO might also explain the difference.

after the injection of heparin. The subsequent part of the curve was smoother

The "half time" of the DAO-differences from the starting level was about 70-80 minutes for both curves. The slopes of the DAO-curve for the other women (each curve was defined by only 2 determinations) showed a half time varying between 40 and 80 minutes

### Discussion

Evidence of a significant secretion of histaminase /DAO from the placenta directly into blood vessels (Swanberg, 1950) does not exclude the possibility of even a fairly considerable transport of this enzyme via the lymphatics from the uterus (for review of the ileo-pelvic aortic lymphatic system, see Nelson *et al.* 1964). Such a substantial transport via the lymph would imply that the concentration of DAO in the thoracic duct lymph were higher than in non pregnant subjects (for comparison see Dahlbäck *et al.* 1968) and higher than in the blood plasma. These two criteria were not observed in the present case. No evidence of any congestion of the lymph (owing to tuberculosis for example) was seen, while the woman was in hospital or during a subsequent 12 month follow up. With reservation for the fact that this obstetrically and gynaecologically healthy pregnant woman had pulmonary changes the DAO-activity in the lymph and that in the blood plasma do not suggest that considerable amounts of the enzyme are transported via the thoracic duct

As far as we know the literature contains no numerical data on the diurnal variation of DAO in the plasma in healthy pregnant women. The spontaneous approximately 5 per cent variation around the 24-hour mean found by us showed that the DAO-increase occurring after the injection of heparin is not appreciably influenced by diurnal fluctuations.

The increase in blood plasma DAO following the heparin injection seemingly occurred sooner in the pregnant women than

Control measurement of waste blood (which was taken between sampling to keep the cannula patent) confirmed that the variation in the activity was true

in men and in non-pregnant women of the same age groups (Hansson *et al.* to be published). This applies particularly to the time of maximum activity which in the 2 women studied most thoroughly occurred about 20 minutes after the injection, compared with 60-120 minutes in men and non-pregnant women. A similar tendency to an early maximum increase of the enzyme was noted also in the other 3 pregnant women, a finding rarely seen in non-pregnant individuals. The heparin-induced increase of the DAO-activity in the blood plasma in the pregnant women did not differ significantly in magnitude from that in non pregnant women (Hansson *et al.* to be published) but was comparatively large. The slope of the DAO-curve was similar for all the pregnant women, and in the 2 women who were followed longest in the group the curves for enzyme activity approached the original level smoothly.

The increase in the DAO activity of the blood plasma disappeared with a half-time of 70-80 minutes in 2 and at approximately the same rate in the other women in the group. Also in this respect the pregnant women resembled the non-pregnant ones in whom the half time varied between 45 and 90 minutes (Hansson *et al.* to be published).

These half-time values differ considerably from the post partum blood plasma findings (Ahlmärk 1944). Even if the pregnancy-initiated histaminase pool that remains in the body after the delivery of the placenta, will retard the decrease in plasma histaminase, the difference found is between a half time of about 75 minutes in the post-heparin group and a half time of 25 to 30 hours in the post-partum group. This difference would presumably have been greater but for the prolonged lymph infusion, rich in DAO (histaminase) in the post-heparin group (Hansson *et al.* 1966 Dahlbäck *et al.* 1968). If the post-heparin DAO enzyme molecule is identical with the pregnancy DAO in blood plasma the different eliminatory rates are an interesting finding. Pregnancy does not markedly influence the elimination of post heparin DAO from plasma. The possibility that there is a puerperal retardation of the elimination of any DAO-activity cannot be ruled out, but the existence of isoenzymes of DAO might also explain the difference.

after the injection of heparin. The subsequent part of the curve was smoother.

The half time of the DAO-differences from the starting level was about 70–80 minutes for both curves. The slopes of the DAO-curve for the other women (each curve was defined by only 2 determinations) showed a half time varying between 40 and 80 minutes.

### Discussion

Evidence of a significant secretion of histaminase /DAO from the placenta directly into blood vessels (Swanberg, 1950) does not exclude the possibility of even a fairly considerable transport of this enzyme via the lymphatics from the uterus (for review of the ileo-pelvic aortic lymphatic system see Nelson *et al.* 1964). Such a substantial transport via the lymph would imply that the concentration of DAO in the thoracic duct lymph were higher than in non pregnant subjects (for comparison see Dahlbäck *et al.* 1968) and higher than in the blood plasma. These two criteria were not observed in the present case. No evidence of any congestion of the lymph (owing to tuberculosis for example) was seen while the woman was in hospital or during a subsequent 12 month follow up. With reservation for the fact that this obstetrically and gynaecologically healthy pregnant woman had pulmonary changes the DAO-activity in the lymph and that in the blood plasma do not suggest that considerable amounts of the enzyme are transported via the thoracic duct.

As far as we know the literature contains no numerical data on the diurnal variation of DAO in the plasma in healthy pregnant women. The spontaneous approximately 5 per cent variation around the 24-hour mean found by us showed that the DAO increase occurring after the injection of heparin is not appreciably influenced by diurnal fluctuations.

The increase in blood plasma DAO following the heparin injection seemingly occurred sooner in the pregnant women than

Control measurement of waste blood (which was taken between sampling to keep the cannula patent) confirmed that the variation in the activity was true.

- a) In thoracic duct lymph and blood plasma from an obstetrically normal pregnant woman with diffuse granulomatous pulmonary changes the enzyme activity of the lymph was the same as that earlier described in men and one non-pregnant woman, while the activity of the blood plasma was 3 times as high as in the lymph i.e. at a level normally found during late pregnancy
- b) In 5 healthy women in the third trimester of pregnancy the blood plasma was examined after intravenous injection of 10,000 LU heparin (conventional dose in anticoagulant therapy of thrombo-embolism) the DAO-activity rose in a similar manner to the reaction non pregnant women, but possibly somewhat faster and rather higher
- c) In the blood plasma from 9 pregnant women, during the daytime spontaneous diurnal fluctuation amounted to about 5 per cent of the mean value.

The investigation produced no evidence of any appreciable transport of DAO from the placenta to the blood plasma via the lymph. The DAO-content of thoracic duct lymph appeared to remain unchanged during pregnancy despite a marked increase of this enzyme in the blood plasma. Neither did the placenta nor the other structures rich in DAO during pregnancy appear to influence significantly the increase in DAO elicited by the heparin.

The spontaneous diurnal variation of the DAO activity in blood plasma during pregnancy will hardly influence the value of a single determination of DAO as a prognostic guide.

#### REFERENCES

- Ahlmark A. *Acta physiol scand* 9 suppl 28 1944  
Dahlback O, Haxson R, Tibbling G and Tryding N. *Scand J clin. Lab. Invest* 20 18 1968  
Dunforth D N and Gorham F. *Am J Physiol* 119 294 1937  
Haxson R, Hohenberg C G, Tibbling G, Tryding N, Westling H and Wetterquist H. *Acta med scand* 180 533 1966  
Kapeller Adler R. *Fed Proc* 24 757 1955  
Lindberg S. *Acta obst et gynec. scandinav* 42 suppl. 1 1953  
2 232 751. *Acta Gyn* 1959



Intravenous injection of heparin in pregnant women thus appears to produce an earlier and possibly but not markedly larger increase of the DAO-activity of the blood plasma than in men and non pregnant women. The activity disappears at approximately the same rate in both pregnant and in non pregnant subjects. The high placental content of DAO thus appears to have but little effect on the increase of enzyme in the plasma following intravenous injection of heparin. The reason for this is obscure. It may imply different kinds of DAO enzymes in man but it may also be explained by the assumption that only a relatively small fraction of the heparin injected reaches the placenta and other DAO rich structures within the pregnant uterus (*Southren et al* 1965). Investigation of the effect of intra arterial injection of small amounts of heparin and determination of the DAO activity on the venous side of the uterine vessels appears to be necessary before anything definite can be said about the role played by the placenta in the increase of plasma DAO after injection of heparin in pregnant women. In this respect the problem may be analogous to that of the role of the placenta in the metabolism of exogenous histamine in pregnancy (*Nilsson et al* 1959 and *Lindberg*, 1963).

The DAO activity of the placenta is several thousand units. The DAO activity is also high in adjacent structures and has been measured relative to that of the placenta with the aid of  $^{14}\text{C}$ -putrescine as substrate (*Southren et al* 1965). Intravenous injection of heparin in a dose of 10–15 000 IU i.e. a conventional single dose in anticoagulant therapy is followed by the liberation of some tens of units of DAO. Our investigation thus produced no evidence that the DAO-content of the placenta is influenced by administration of heparin. But even if all the DAO liberated derived from the placenta the local decrease in the concentration of the enzyme would still be only about one per cent or less.

### SUMMARY

The activity of diamine oxidase (DAO) /histaminase (E. C. 1.4.3.6) was studied in pregnant women and the following observations were made.

## DIAMINE OXIDASE (HISTAMINASE) IN BLISTER FLUID AND BLOOD PLASMA DURING PREGNANCY

BY

ROY HANSSON

The increased histaminolytic activity of human blood plasma during pregnancy (Marcou *et al* 1938 Ahlmark 1944) has been ascribed to the activity of an enzyme histaminase, (Best 1929 Best and Mc Henry 1930) which catalyses the oxidative deamination of among other substrates histamine. It has been discussed (e.g Kapeller Adler 1965 and Zeller 1965) whether histaminase really is identical with diaminoxidase (DAO) (Zeller 1938) but both names are used synonymously (Report of the Commission on Enzymes of the International Union of Biochemistry Oxford, Pergamon 1961) for diamine oxygen oxidoreductase (deaminating) E.C No 1.4.3.6. The origin of the increase of histaminase in the blood plasma has been traced to the decidual part of the placenta (Swanberg, 1950) The relative distribution of the enzyme in human maternal and foetal blood plasma as well as in various trophoblastic and decidual tissues at parturition has recently been studied with the use of a  $^{14}\text{C}$ -putrescine method (Sonahven *et al* 1965) But apart from the report of a single case (Hansson Trydling and Törnqvist in press) where thoracic duct lymph from a pregnant woman was found to contain approximately the same concentration of DAO activity as that found in non pregnant women and in only one third of the concentration of that found in the woman's blood plasma, little is known about the distribution of the enzyme in the other components of the fluid phase during pregnancy It was recently reported (Hansson Kitzala Rorsman and Trydling, 1967) that fluid in suction blisters produced by the method of

- Marcou I, Atlanastiu-Vergu E, Chiricdanu D, Cosma G, Gingold N and Parhon C. C. Presse. méd 46 371 1938
- Nelson J, Masterson J, Herman P and Benninghoff D. Amer J Obstet. Gynec. 88 460 1964
- Nilsson K, Lindell S E, Schayer R. W and Westling, H. Clinical Science 18 313 1959
- Southren A. L., Kobayashi Y, Brenner P and Weingold A. B. J Appl. Physiol 20 1048 1955
- Southren A. L., Kobayashi Y, Jung, W, Carmody N C and Weingold A. J Clin Endocr Metab 26 1005 1966
- Svanberg, H. Acta physiol scand 23 suppl 79 1950
- Trydning, N. Scand. J clin Lab Invest., 17 suppl. 86 195 1955 a
- Ibidem Scand. J clin. Lab Invest., 17 suppl 85 197 1965 b

Received on Feb 22, 1968

Table I

Subject no	1	2	3	4	5	
Age (years)	70	21	22	28	33	
Pregnancy no	III	I	II	I	IV	
Pregnancy weeks	35	31	26	29	33	
Plasma DAO before (U/l)	999	522	111	178	227	
after	918	350	116	142	219	
mean	95	54	114	180	223	
Blister DAO (U/l)	122	376	147	181	258	
Plasma protein g per 100 ml mean	6.88	6.81	7.24	6.40	6.03	
Blister protein g per 100 ml	1.37	1.30	1.48	1.53	1.27	
Blister albumin	87.9	60.5	78.4	83.9	71.2	
Rela- tive	$\alpha_1$ -globulin	3.6	5.8	3.3	2.4	4.2
	$\alpha_2$ -globulin	4.4	5.8	3.0	2.6	3.8
per cent	$\beta$ -globulin	9.5	7.7	7.2	7.6	10.1
	$\beta_2$ -globulin	2.2	3.7	1.5	0.8	3.1
age	$\gamma$ -globulin	12.4	16.5	6.6	2.9	7.6
Scintillation time before blistering, minutes	124	75	95	150	140	

l-pyrroline is almost quantitatively (more than 96 per cent) extracted from the water phase by washing it twice with 10 ml of toluene (in which the scintillators are already dissolved) whereas less than 0.2 per cent of the remaining  $^{14}\text{C}$ -putrescine (often less than 0.1 per cent) is contaminant in the resulting 20 ml of scintillator fluid. After each washing the water phase is frozen and the toluene phase decanted into a scintillator vial for counting (Packard Tricarb 2000-series). Sample blanks are prepared in the way described above but not incubated. With an incubation time of 120 minutes and a sample amount of 50  $\mu\text{l}$  the response is linear up to an enzyme activity of 3  $\mu\text{moles}$  of putrescine per minute and litre ( $\text{L} = 3 \text{ U/l}$ ). When more active specimens are measured it is convenient to use a shorter incubation time and/or dilution of the sample. The normal blood plasma DAO-activity ranges from 0 to 25 mU/l in non-pregnant individuals (the same for both sexes). In pregnancy a rise will occur to values between

*Kiistala and Mustakallio* (1964) in non pregnant volunteers contained DAO in a concentration at least as high as that in the blood plasma in spite of the fact that the protein content was only about one third of that in the latter. Injection of heparin was followed by an increase of DAO but the increase was only a fraction of that which occurred in the blood plasma.

In order to assess the possibility of ultrafiltration of DAO from the blood plasma into suction blisters the protein content and enzyme activity of the fluid from such blisters in pregnant women were determined and compared with the corresponding concentrations in the plasma.

### *Material and Methods*

Samples of venous blood were obtained from 5 healthy non fasting pregnant women immediately before the test. Further information on the volunteers is given in Table I. The blisters were produced by the method of *Kiistala and Mustakallio* 1964. The proximal area of the skin on the volar aspect of the (left) lower arm was washed with diluted alcohol and allowed to dry in the air. The suction cup of the angiotonometer was applied. A negative pressure of 200–250 mm Hg was produced and maintained long enough to produce blisters from which altogether about 200  $\mu$ l of fluid could be obtained. The negative pressure was then gradually diminished and the cup and the apparatus were removed. With a thin sterile cannula and a plastic syringe the blisters were punctured and their content was transferred to a microcentrifuge tube made of polypropylene (Sanz model). Samples of venous blood were obtained immediately before and after the blisters had been emptied.

The DAO-activity was estimated according to the principles of *Okuyama and Kobayashi* (1961) in a modification for ultra micro-samples by *Trydting* (1965).  $^{14}$ C putrescine SA 1 6 Ci/mole 1 mM, in 1/15 M phosphate buffer pH 7.4 and an equal volume (50  $\mu$ l) of blister fluid or blood plasma are incubated in 1.90 ml 1/15 M phosphate buffer pH 7.4 at 37 °C for 120 minutes and, thereafter immediately chilled to about 0 °C by immersion in a cold (–15 °C) bath. The reaction product,  $^{14}$ C

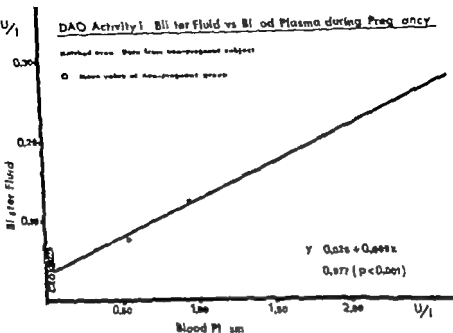


Fig. 1.

### Discussion

The blisters that form under the suction cup of the angiotonometer have no direct point of contact with the apparatus. In our series the longest interval between formation of the blister and puncture was 30 minutes. The concentration of the sodium and that of the potassium in the blister fluid were identical with the normal concentration in plasma/intercellular fluid. This suggests that the cell membranes had not been damaged, but it does not exclude the possibility of a very rapid electrolyte shift between the blister and interstitial fluids being able to eliminate any differences that might have occurred. No appreciable difference was found in the blistering tendency among the pregnant women, compared with previously examined non-pregnant volunteers. The concentration of the protein in the blisters was fairly uniform in all the women. In the previous investigation (Hansson

0.9 and 8 U/l. The coefficient of variation of the procedure, as calculated from a series of double determinations with Dahlberg's formula ( $S.D. = \sqrt{\sum J^2 / (2n)}$ ) and  $\text{coeff var} = S.D. / (\text{mean value of series})$  is between 15 and 30 per cent in the lower normal range of DAO-activity and in the pregnant group less than 2 per cent.

The protein concentration in the blister fluid was determined by the Waddell method (1956) and in blood plasma by the biuret method. Paper electrophoresis was performed by the method of Laurell (Laurell and Skoog (1956)). The alkaline cations were determined by flame photometry (Eppendorf) in the pooled surplus blister fluid (in 10 to 40  $\mu$ l portions).

### Results (see Table I)

The mean protein content of the blood plasma was found to be 6.7 (Range 6.0–7.2) and that of the fluid from the blister 1.4 (Range 1.3–1.5) g per 100 ml. Paper electrophoretic separation of the blister content showed the following pattern:

Albumin	72.4 % (Range 60.4–83.9) of the protein	
$\alpha_1$ -globulin	3.9 % (Range 2.4–5.8)	"
$\alpha_2$ -globulin	3.9 % (Range 2.6–5.8)	"
$\beta_1$ -globulin	8.4 % (Range 7.2–10.1)	
$\beta_2$ -globulin	2.2 % (Range 0.6–3.7)	
$\gamma$ -globulin	9.2 % (Range 2.9–16.5)	"

The concentrations of sodium and of potassium in the blister fluid (triplicate determinations) were 140 and 4.55 mEq/l respectively. The DAO-concentration in the blood plasma at the beginning of the test did not differ significantly from that found at the end of the test.

The concentration of DAO in the blister fluid was positively correlated with the corresponding activity in the blood plasma (Fig. 1).

In two volunteers studied, the blister content together with an equal amount of blood plasma was found to possess the same enzyme activity as the sum of the activities in the individual samples determined separately. DAO-inhibitor could thus not be demonstrated in the blister fluid as compared with blood plasma.

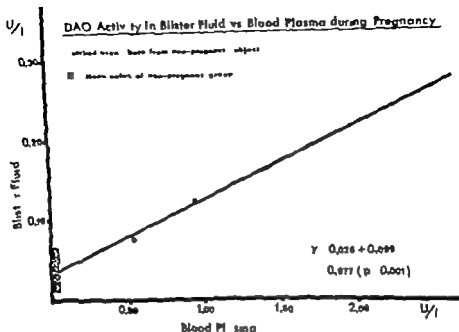


Fig. 1

### Discussion

The blisters that form under the suction cup of the angiotensinometer have no direct point of contact with the apparatus. In our series the longest interval between formation of the blister and puncture was 30 minutes. The concentration of the sodium and that of the potassium in the blister fluid were identical with the normal concentration in plasma/intercellular fluid. This suggests that the cell membranes had not been damaged, but it does not exclude the possibility of a very rapid electrolyte shift between the blister and interstitial fluids being able to eliminate any differences that might have occurred. No appreciable difference was found in the blistering tendency among the pregnant women, compared with previously examined non-pregnant volunteers. The concentration of the protein in the blisters was fairly uniform in all the women. In the previous investigation [Hansson



*et al* 1967) the protein concentration varied but in that series the subjects differed in age and the application site of the suction cup and the total size of the blisters varied much more than in the present series. The intercellular fluid is not synonymous with lymph from the same area especially regarding the concentration of protein, which in the lymph vessels has been found to be higher than in the extravascular interstitial space (Földi 1967). The concentration of protein in blister fluid is somewhat lower than that given for the interstitial fluid of the dermis (Schultze and Heremans 1966) but higher than that in oedematous fluid in cardiac oedema and is of the same order as in lymphoedematous interstitial fluid. The paper electrophoretic pattern suggested only a protein-containing ultrafiltrate and did not differ significantly (Student's *t* test) from findings in non pregnant women (Hansson *et al* 1967). Therefore it would appear that pregnancy is not accompanied by any appreciable change in the relative composition of the protein in blister fluid. The DAO activity of blister fluid proved to be clearly dependent on the concentration of the enzyme in the blood plasma. The regression line intersected the normal range (Fig 1 hatched area, Hansson *et al* 1967) near the mean value found for non pregnant women. The DAO-content of the blister fluid may thus have a component not dependent on the DAO-content of the blood plasma and secondly a component of much lower concentration than, but correlated with that in the blood plasma. In non pregnant individuals the activity of DAO was on the average about 1.7 times as high in blister fluid as in plasma and much higher (about 5 times) if the enzyme activity was correlated with the protein concentration (Hansson *et al* 1967). The plasma-correlated component of DAO was about 10 per cent of that in the plasma in our comparatively small group of volunteers. If the activity of this enzyme in blister fluid were correlated with the protein concentration and the same manoeuvre performed with DAO and protein concentrations in plasma the resulting specific activities in the two fluids would be more similar. The relative DAO-content of the blister fluid then was about half of that in the plasma.

The suction blister and its fluid are an artefact. The electrolyte

concentration, protein content and electrophoretic pattern suggest that the negative pressure sucks the fluid (interstitial fluid and lymph?) out of the dermis without any appreciable injury to the cell membranes. An alternative or associated explanation is that the fluid leaves the blood stream by ultrafiltration.

In a previous study it was found that thoracic duct lymph from a pregnant woman contained 0.5 U DAO/l, while the blood plasma contained DAO in a concentration 3 times higher (Hansson Tryding and Törnqvist in press). Since the concentration of the enzyme in the lymph was of the same order as in men and a non-pregnant woman (Dahlbäck *et al.* 1968) this finding was thought to suggest that the lymph vessels do not play any appreciable role in the transport of DAO into the blood stream in pregnant women. Neither does the interstitial fluid and lymph as a "DAO-compartment" appear to have any great quantitative importance during pregnancy.

Our present findings indicate that the tendency of DAO to be ultrafiltered out of the blood stream is relatively small. In the literature the DAO is usually described as an enzyme activity but no definite information is available about its molecular size or about its isoenzymes if any. Therefore our findings do not warrant any conclusions regarding the ultrafiltrability of the enzyme as a manifestation of the behaviour of a homogenous molecule. One might, however, imagine the possibility of one component formed by or liberated from the local tissue (distally to or immediately under the suction cup). The behaviour of the second component requires simultaneous examination of lymph or interstitial tissue from the limbs and of blister fluid to find out whether ultrafiltration occurred *in situ* or whether a small increase of DAO due to the increase in the plasma during pregnancy really occurs also in the interstitial fluid.

### SUMMARY

During pregnancy blister fluid, obtained by the technique of Hässala and Mustakallio shows the same protein pattern after paper electrophoretic separation as in non-pregnant individuals.

The diamine oxidase (DAO)-activity however is higher than

in non pregnant persons and shows a positive correlation with the plasma DAO activity. The enzyme activity is about one tenth of that in plasma. But without known correlation to the plasma enzyme level there seems to be some blister fluid DAO-activity of the same magnitude as in non pregnant individuals. The findings do not indicate that during pregnancy the extra vascular intercellular compartments store large amounts of DAO as compared with the intrauterine structures and the blood plasma.

## REFERENCES

- Ahlmark A. *Acta physiol scand* 9 suppl. 28 1944  
 Best C H J *Physiol (Lond)* 67 256 1929  
 Best C H and Mc Henry E. W. *J Physiol (Lond.)* 70 349 1930  
 Dahlbäck O, Hansson R, Tibbling G and Tryding, N. *Scand J clin. Lab Invest.* 21 000 1958  
 Földi M. *Experientia Basel suppl.* 14 (ed. Collette J M, Janet G and Schoffeleers E.) 11 1967  
 Hansson R, Kilstala U, Rorsman H and Tryding, N. *Acta dermat. venerol* 47 94 1967  
 Hansson R, Tryding, N and Törnqvist A. *Acta obstet. gynec. scand.* 48 8 1969  
 Kapeller Adler R. *Fed. Proc.* 24 757 1965  
 Kilstala U and Mustakallio K. K. *Lancet* 1 1444 1964  
 Laurell C B, Laurell S and Skoog, N. *Clin Chem.* 2 99 1956  
 Marcou J, Athanasiu-Vergu E, Chiriacanu D, Cosma G, Gingold N and Parhon C C. *Presse méd.* 46 371 1938  
 Okuyama T and Kobayashi Y. *Arch. Biochem. Biophys.* (N Y) 95 242, 1961  
 Schultz H E and Horemans J F. *Molecular Biology of Human Proteins* Elsevier Publishing Company Amsterdam-London-New York 1966  
 Southren A L, Kobayashi Y, Brenner P and Weingold A. B. *J Appl. Physiol* 20 1948 1935  
 Swanberg, H. *Acta physiol scand.* 23 suppl 79 1950  
 Tryding, N. *Scand. J clin. Lab Invest* 17 suppl 86 196 1965  
 Waddell W J. *J Lab Clin. Med.* 48 311 1956  
 Zeller E. A. *Helv Chim Acta* 21 880 1938  
 - *Fed. Proc.* 24 766 1965

Received on Feb 22 1968

## THE $^{125}\text{I}$ TRIIODOTHYRONINE-RESIN UPTAKE TEST DURING LABOUR

BY

O. CASTRÉN, L. LAAKSO AND T. NIKKARI

The size of the unsaturated portion of plasma proteins binding the thyroid hormones (mainly thyroxine-binding globulin, i.e. TBG) can be studied by means of the  $^{125}\text{I}$  triiodothyronine uptake test ( $\text{T}_3$ -test) the smaller the uptake the larger the unsaturated portion, and *vice versa*. Since the experiment is performed *in vitro* it can also be used to examine the thyroid status during pregnancy without detriment to the foetus. Assessment of the results, however, is difficult, because the size of the unsaturated portion of TBG depends both on the thyroid hormone content and on the amount of TBG itself (Sisson 1965). Low  $\text{T}_3$ -uptake test values are seen in hypothyroidism, the amount of thyroid hormones in the plasma being reduced. During pregnancy the  $\text{T}_3$ -uptake drops apparently as a result of the increased total capacity of TBG (Robbins and Rall 1960) the PBI increases for the same reason (Hamolsky *et al.* 1959). An increase of TBG is seen 3-4 weeks after ovulation (Dowling *et al.* 1956 Hamolsky *et al.* 1959) as is the fall in  $\text{T}_3$ -uptake. The  $\text{T}_3$ -uptake is at its lowest level in the 2nd to 3rd trimester when it is 60-80 per cent of the corresponding value of the non-pregnant woman (Spafford *et al.* 1960 Clark and Horn 1965). Its restoration to the normal level occurs one to two weeks *post partum* (Hamolsky *et al.* 1959) although the TBG does not reach its normal level until the fifth *post partum* week (Dowling *et al.* 1956). Preliminary studies showed that changes also occur in the red cell

uptake of  $^{131}\text{I}$  labelled  $\text{T}_3$  during labour (Laakso 1964). The purpose of the present study was to shed additional light on these changes.

### Material

The series comprised 15 parturients with no symptoms of toxæmia of late pregnancy. Venous blood samples were drawn into heparinised test tubes as follows:

A = 7 days before the calculated term,

B = on the parturient's admission to hospital, when labour had started but contractions were still irregular,

C = during labour the uterine os 5–7 cm open,

D = immediately *post partum* before expulsion of the placenta,

E = 2–3 hours *post partum*,

F = 3 days *post partum*,

G = 5 days *post partum*,

H = from the umbilical cord at the moment of division.

Cæsarean section were performed in cases 14 and 15 because of contracted pelvis. At the time of operation, the contractions were still irregular and the uterine os 2–4 cm open.

The control series consisted of five euthyroid non-pregnant women of fertile age; their blood samples were taken in the premenstruum.

### Method

The resin  $\text{T}_3$ -uptake was measured using the method of Woldring *et al.* (1961). The plasma was separated from the cells by centrifugation. To 20 mg of the resin (Amberlite CG-50 type II) in 2 cc physiological saline solution was added 0.1 cc radioactive  $^{131}\text{I}$  triiodothyronine ( $\text{T}_3$ ) (about 0.01  $\mu\text{g}$ ) and 0.5 cc of the plasma to be investigated. The mixture was incubated in a water bath at 37 °C for 30 minutes.

After incubation, the total radioactivity was measured in a well type scintillation counter. The tubes were then centrifuged, supernatant liquid removed and the resin washed and recentrifuged five times with saline solution. After these washings the tubes were recounted to determine the resin uptake of radio-

Table 1.  $T_3$ -uptake ratios (multiplied by 100) of plasma samples taken A. before labour B. during irregular uterine contractions C. during labour (in cases 14 and 15 immediately before operation) D. immediately post partum, E-G 2, 3 hrs, 3 and 5 days post partum respectively H. from the cord blood

Patient	A	B	C	D	E	F	G	H
1		74	83	73	72	89		116
2		74	78	76	54	78		
3			90	87	93	102		103
4		88	92	85	78	98		132
5		74	67	60	72	84		89
6		62	83	56	54	96		87
7		75	88	78	60	74		92
8	81		94	85	73	79	88	108
9	78		78	78	80	83	88	96
10	77	80	82	77	74	75	90	102
11	74		73	80	79	78	82	88
12	67	76	86	79	74	83	87	94
13	67	70	74			72	74	
	6	9	13	12	12	13	6	11
M	75	75	82	76	72	81	85	101
SD	$\pm 7.9$	$\pm 7.0$	$\pm 8.0$	$\pm 9.4$	$\pm 11.2$	$\pm 11.6$	$\pm 5.9$	$\pm 13.8$
SEM	$\pm 2.9$	$\pm 2.3$	$\pm 2.2$	$\pm 2.7$	$\pm 3.2$	$\pm 3.2$	$\pm 2.3$	$\pm 4.1$
14		88	95	84		101		
15		88	115	91		100		

Abbreviations: n. number of samples; M. mean; SD. standard deviation; SEM. standard error of the mean.

activity. The uptake was calculated as a percentage of the original radioactivity (=  $T_3$ -uptake percentage). To render the values obtained at different times comparable with each other the uptakes were also expressed as a ratio to the simultaneous resin uptake of the control sera (resin uptake ratio Clark and Horn 1965).

### Results

The results are presented in Table 1. It is seen that the  $T_3$ -uptake ratio before and during labour was significantly lower than in

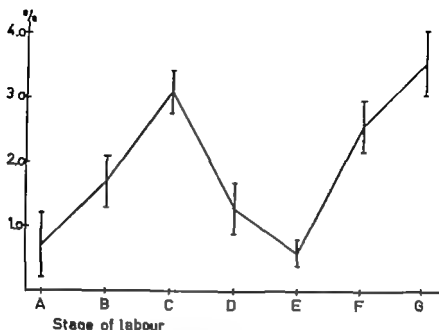


Fig. 1. Mean difference of the  $T_3$ -uptake percentages at various stages of labour from the minimum  $T_3$ -uptake values. The standard deviations of the differences are given by vertical segments.

the non pregnant women ( $p < 0.001$ ). A week before the calculated term and as long as the contractions were irregular (A-B) the  $T_3$ -uptake ratio was only about 75 per cent of the control value. By the time the contractions were regular and the uterine os was 5-7 cm open (C) the  $T_3$ -uptake ratio increased to an average of 82 per cent, to fall again, in the sample drawn immediately *post partum* (D) to 76 per cent of the control value; the lowest value, 72 per cent of the control value, was obtained in the present study 2-3 hours *post partum* (E). Subsequently the  $T_3$ -uptake ratio increased gradually, although on the fifth *post partum* day (G) it was still 15 per cent lower than the control value.

Since the  $T_3$ -uptake was found to fluctuate during labour, the authors wished to discover whether the changes might be attributable to the strain produced by labour or to the contractions of the uterine muscle. To exclude these factors, samples were drawn from two patients who, because of a contracted pelvis

had to be treated by Caesarean section in an early phase of labour (Cases 14 and 15). The mean resin uptake ratios of these patients in the different phases of parturition (Table I) were found to be as follows: B 88 C 105 D 87 F 100 per cent. These changes, although not so marked were parallel to those found during normal labour.

The mean  $\text{T}_3$ -uptake of the blood sample taken from the umbilical cord was the same as in the control series.

To obtain a better idea of the changes in  $\text{T}_3$ -uptake in the course of labour the lowest percentage obtained for each parturient (most frequently at stages D-E) was subtracted from the  $\text{T}_3$ -uptake percentages obtained in the various stages of labour. Fig. 1 shows the resulting mean differences from the minimum values. It should be noted that the time intervals, seemingly equal on the abscissa, were not in reality of equal duration. The  $\text{T}_3$ -uptake so calculated was found to increase from A to C highly significantly ( $p < 0.01$ ) to fall from C to E very highly significantly ( $p < 0.001$ ) and to increase again from parturition to the fifth post partum day very highly significantly ( $p < 0.001$ ). Consequently the changes in the course of labour cannot be considered accidental: they must have been produced by some factor associated with the labour.

### Discussion

In agreement with earlier findings the present study showed that the  $\text{T}_3$ -uptake towards the end of pregnancy was only 75 per cent of the values obtained on non-pregnant women, and the normal value had still not been reached five days post partum.

No attention has been given previously to the changes in  $\text{T}_3$ -uptake during labour. The rise and fall of the  $\text{T}_3$ -uptake recorded in the present study in the course of a few hours and in conjunction with parturition, are difficult to explain. The  $\text{T}_3$ -uptake is known to be increased in hyperthyroidism, in which the ratio of thyroid hormone and TBG is elevated. During pregnancy the capacity of serum binding proteins, especially TBG is increased, but no changes in these parameters have been shown during labour. Thyroxin crosses the placenta (Pickering *et al.* 1958)



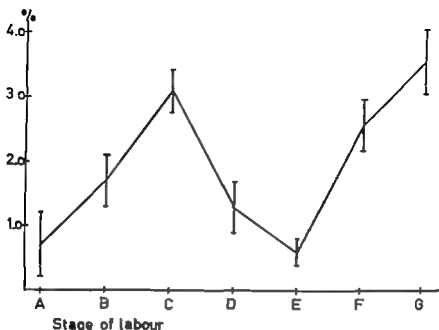


Fig. 1. Mean difference of the  $T_3$ -uptake percentages at various stages of labour from the minimum  $T_3$ -uptake values. The standard deviations of the differences are given by vertical segments.

the non pregnant women ( $p < 0.001$ ). A week before the calculated term and as long as the contractions were irregular (A-B) the  $T_3$ -uptake ratio was only about 75 per cent of the control value. By the time the contractions were regular and the uterine os was 5-7 cm open (C) the  $T_3$ -uptake ratio increased to an average of 82 per cent, to fall again in the sample drawn immediately *post partum* (D) to 76 per cent of the control value. The lowest value, 72 per cent of the control value, was obtained in the present study 2-3 hours *post partum* (E). Subsequently the  $T_3$ -uptake ratio increased gradually although on the fifth *post partum* day (G) it was still 15 per cent lower than the control value.

Since the  $T_3$ -uptake was found to fluctuate during labour the authors wished to discover whether the changes might be attributable to the strain produced by labour or to the contractions of the uterine muscle. To exclude these factors samples were drawn from two patients who because of a contracted pelvis

thereafter slowly (81 per cent on the 3rd and 85 per cent on the 5th day post partum)

In samples of umbilical cord blood the mean  $\text{T}_3$ -uptake was 101 per cent of the value for the non-pregnant women.

The factors hitherto known to change the capacity of the serum proteins to bind thyroid hormone, and therefore also the  $\text{T}_3$ -uptake, were discussed. The rapid changes found in the present study could not be explained on the basis of such factors alone.

#### REFERENCES

- Clark F and Horn D *J Clin Endocr* 25 39 1965  
Downing J T, Fretwell N and Ingber S *J Clin. Invest* 35 1263 1956  
*J Clin. Invest.* 39 1119 1960  
Egstrup N and Engström W *J Clin. Endocr* 19 783 1959  
Goddan J L and Gernert E S *J Endocrinol* 29 167 1964  
Hawelsky M V, Golodetz A and Friedberg, A S. *J Clin. Endocr* 19 103 1959  
Hollander C, Garcia A, Strauss S and Selenkow H *New Engl. J Med.* 269 501 1963  
Horster F and Klein E *Dtsch. med. Wochs.* 89 983 1964  
Ludso L Unpublished data 1954  
Marik J F *J Clin. Endocr* 25 852, 1965  
Marik J F, Wolfson J and Klein R *J Paediat.* 58 32, 1961  
Pichler, D, Konstantis N, Benton R and Merchant R *Am. J Dis Child.* 95 616 1958  
Robbins J and Rall J *Physiol Rev* 40 413 1960  
Sisson J C *J Nucl Med* 6 853 1965  
Spafford, N, Carr E, Lowrey G and Birmaher W *Am J Dis Child* 100 844 1950  
Woldring, M G, Balch A and Doorenbos H *Acta Endocrinol* 37 607 1961

Received on Feb. 12, 1968

and high free thyroxin values have been recorded in the newborn (Marks 1965). The  $T_3$ -uptake of the newborn, however, has been reported to be reduced (Spafford *et al.* 1960) or increased (Marks *et al.* 1961) or as in the present study it has been found to be normal. In any case foetal changes are hardly likely to be sufficient to account for the maternal variations.

In nephrotic patients the  $T_3$ -uptake increases because there is a loss of proteins including TBG (Robbins and Rall 1960).

Administration of oestrogens reduces the  $T_3$ -uptake (Engbring and Engström 1959; Dowling *et al.* 1960) a finding that might explain the low values recorded during pregnancy. Similarly chorionic gonadotropin secreted after hysterectomy by chorionic carcinoma metastases reduces the  $T_3$ -uptake (Godden and Garnett 1964). Steroids inhibiting ovulation have the same effect (Hollander *et al.* 1963) which is also demonstrable in the premenstruum (Horster and Klein 1964).

The changes listed above in maternal or foetal thyroid function or perhaps in placental function during labour and the accompanying changes in oestrogen or gonadotropin secretion can hardly account for the relatively rapid changes found in  $T_3$ -uptake during labour. It is apparent that the binding sites of thyroid binding protein are transiently reduced in the course of parturition for a reason to be ascertained by later studies.

## SUMMARY

$T_3$ -uptake tests were performed on 15 normal parturients. The samples were taken one week before the calculated term in different stages of labour and *post partum*. A series of euthyroid non pregnant women acted as controls. The  $T_3$ -uptake at the end of pregnancy just before parturition was 75 per cent of the control level.

While the contractions were regular during labour and when the uterine os was open, the  $T_3$ -uptake rose to 82 per cent of the control values and fell to the pregnancy level in the sample taken immediately *post partum*. The uptake was at its lowest 2-3 hours *post partum* (72 per cent of the control level) and increased

3.000–4.000 g) and that of the controls 3.840 g (range 3.040–5.150 g). The experimental conditions were the same as in earlier studies (Gelli *et al.* 1966 and 1968). Thirteen rabbits were given an i.v. infusion of 30 % glucose solution in the course of 6 hours on the 29th day of pregnancy. The infusion was given through a polyethylene catheter introduced into an auricular vein and advanced to the vena cava. Shortly after the infusion, the mother was sacrificed by intracasternal injection of 2 ml of a 2 % Xylocaine® solution. The foetuses were removed via laparotomy. One foetus was placed in a bath of liquid paraffin (37 °C) and the ECG recorded. The remaining foetuses in each litter were placed with intact membranes in a thermostat bath (37 °C) of physiological saline; they were thus made anoxic. As long as heart activity could be recorded by ECG (Gelli and Gyulai 1968) foetuses were taken at irregular intervals for biochemical analyses. Before sampling, the occurrence of heart activity was checked.

The controls consisted of foetuses whose mother had not been given a glucose infusion but were otherwise treated identically to those of glucose-loaded mothers. As a rule, one litter from a glucose-loaded mother and one from an untreated mother were studied concurrently.

Foetal blood was obtained by heart puncture. The blood was carefully and slowly aspirated in order to avoid formation of bubbles and haemolysis. It was then transferred to a microlitre test tube which was centrifuged for 2 minutes at 15 000 r.p.m. The plasma was separated off and kept deep-frozen for electrolyte determinations. A separate blood sample was taken for determination of lactate. This blood was precipitated within 20 seconds with 30 % trichloroacetic acid (TCA) and the lactate determined according to Barker and Summerson (1941). Micro-methods were used for determination of blood pH, total CO<sub>2</sub>, potassium, chloride, calcium, total protein, inorganic phosphorus and lactate (Sanz 1957 and 1962; Thalme 1962, 1963, 1964 and 1967).

Samples from the apex of the heart were taken for electrolyte determinations. Potassium and magnesium were determined with atomic absorption flame photometry (Beronius, Bergström and Hultman 1968). The muscle samples were dried at 90 °C and

Gelli, M. G. Bergström, J. Hultman E. and Thalme B. *Acta obst. et gynec. scandinav.* 48: 34, 1969  
From Karolinska Institutet the Department of Obstetrics and Gynaecology (Prof. A. Ingelman-Sundberg) Sabbatsberga Sjukhus and the Department of Paediatrics (Prof. J. Lind) Karolinska Sjukhuset the Renal Clinic (Doc. H. Bucht) and the Central Laboratory (Prof. B. Josephson) St. Eriks Sjukhus Stockholm Sweden

## HEART MUSCLE AND PLASMA ELECTROLYTES IN NORMAL AND GLUCOSE LOADED RABBIT FOETUSES UNDER ANOXIA

BY

M. G. GELLI, J. BERGSTRÖM, E. HULTMAN AND B. THALME

In earlier studies in rabbit foetuses we showed that intravenous infusion of glucose in the mother on the 29th day of pregnancy raised the glycogen content of the heart in the foetuses and that their heart activity had a longer duration under anoxia than that in control foetuses whose mother had not received glucose (Gelli *et al.* 1966 and 1968). Of all the parameters measured—i.e. heart muscle glycogen, liver glycogen, blood glucose and blood pH—the raised heart muscle glycogen content appeared to be the only factor that could explain the prolongation of heart activity. It is however known that the electrolyte conditions both extra and intracellularly have a great influence on the heart activity (Rona, Kalin and Chappel 1965; Selye and Gabblani 1965). It is also known that the cells lose potassium to some extent under anoxia (Calkins, Taylor and Hastings 1954).

The object of the present experiments was to define in greater detail the influence of these factors.

### *Material and Methods*

The material consisted of 28 pregnant rabbits of local breed; the average weight of the experimental animals was 3 730 g (range

tein concentration using the following formula (Eisenman Mac kenzie and Peters, 1936)

$[H_2O] = 984 - 7.18 \text{ protein concentration (g/100 ml plasma)}$

Excess sodium ( $Na_e$ ) i.e. the quantity of  $Na$  not present in the chloride space was calculated as follows

$$Na_e = Na_m - \frac{[Na] H_2O_{Cl}}{1000}$$

where  $Na_m$  = the sodium content of muscle in mEq/100 g FFS and  $[Na]$  = the extracellular (plasma) sodium concentration in mEq/L

Similar calculations were made of  $K_e$  i.e. the quantity of  $K$  present outside the chloride space in the muscle.  $H_2O_e$  i.e., the quantity of water not present in the chloride space was calculated as  $H_2O_m - H_2O_{Cl}$

It is true that the chloride space cannot be regarded as a satisfactory measure of the quantity of extracellular water since varying amounts of chloride are present intracellularly in different cells. The real extracellular quantity of water in muscle must therefore be less than the chloride space. We nevertheless calculated the size of this space and used it as the highest possible value of extracellular water. This made it possible to calculate a highest possible value for the extracellular proportion of a given electrolyte in muscle and consequently by subtraction of this amount from the total tissue content, a lowest possible value for the intracellular quantity of the relevant electrolyte (denoted here as excess  $K$  ( $K_e$ ) excess  $Na$  ( $Na_e$ ) etc.) If an increase in a certain electrolyte e.g.  $K$  is considerably greater than the maximally possible extracellular proportion—i.e., there is a small difference between total  $K$  and excess  $K$ —one can therefore conclude that an intracellular increase has occurred. Excess water was interpreted according to similar principles.

## Results

### Plasma electrolytes

The individual values of plasma pH,  $K$ , lactate,  $Na$ ,  $Cl$ ,  $PO_4$ ,  $Ca^{++}$ , protein and total  $CO_2$  in the controls and experimental foetuses during anoxia are given in Figs. 1-8

the fat extracted with petroleum ether. In 5 experimental and 5 control litters duplicate muscle samples were weighed on a Cahn electrobalance both before and after drying, as well as after extraction of fat using a method previously described (Bergström 1962) and the water content was determined.

In the cases where duplicate samples were obtained one sample was taken for determination of sodium, chloride, potassium and phosphorus by neutron activation analysis (Bergström 1962). The neutron irradiations were performed in reactor R 1 at AB Atomenergi, Stockholm. The samples from one litter were generally irradiated together as close as possible to reduce the influence of neutron flux variations. Good agreement was found between duplicate potassium determinations with activation analysis and absorption flame photometry.

Samples for lactate determination were taken from 12 litters. These muscle samples were frozen directly in liquid nitrogen and then freeze-dried. The freeze-dried sample was analyzed with an enzymatic method (Scholte *et al.* 1959). The results were expressed in mmol/100 g dry weight.

The values obtained for water and electrolytes were expressed with 100 g fat free substance (FFS) as reference base.

The chloride space *i.e.* the distribution volume for chloride under the assumption that the chloride concentration in this space is the same as in the extracellular fluid was calculated according to the following formula:

$$H_2O_{Cl} = \frac{[Cl]_m \cdot 1000}{[Cl]}$$

where  $H_2O_{Cl}$  = the chloride space in ml,  $[Cl]_m$  = the chloride content in mEq/100 g FFS and  $[Cl]$  = the extracellular chloride concentration in mEq/l.

The extracellular chloride concentration was calculated from the plasma chloride concentration  $[Cl]_p$  and plasma water  $[H_2O]_p$  and a Donnan factor of 0.96 according to the following formula:

$$[Cl] = \frac{[Cl]_p \cdot 1000}{0.96 [H_2O]_p}$$

The plasma water (ml/l plasma) was calculated from the pro-

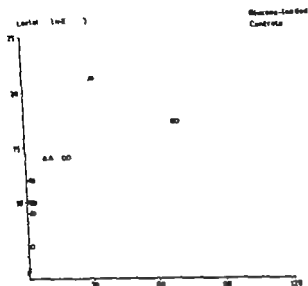


Fig. 3. Blood lactate during anoxia.

pH (Fig. 1) Blood pH fell successively in both groups. The values in the glucose-loaded foetuses tended to be somewhat lower than in the controls at the end of anoxia.

K (Fig. 2) A rise in the plasma potassium occurred in both groups, and was significantly higher in the control foetuses than in the glucose-loaded foetuses in the intervals 0-10 min ( $P < 0.01$ ), 11-30 min ( $P < 0.01$ ) and 31-50 min ( $P < 0.01$ ).

Lactate (Fig. 3) The lactate concentration increased greatly in both groups. The main increase occurred during the first 50 minutes. There was a significant difference between the mean values obtained at 0-10 min and 50-70 min ( $P < 0.001$ ).

Na and Cl (Fig. 4) The levels of sodium and chloride remained fairly stable during the time of the experiment.

PO (Fig. 5) During anoxia the concentration of inorganic phosphorus in plasma rose successively. The increase was significant ( $P < 0.001$ ) with no difference between the groups.

Ca (Fig. 6) The plasma calcium remained fairly unchanged in both groups during anoxia.



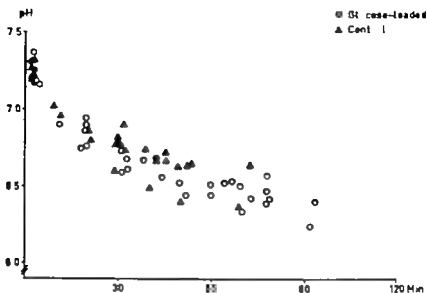


Fig. 1 Blood pH during anoxia.

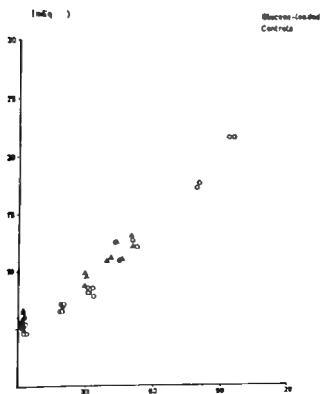


Fig. 2. Plasma potassium during anoxia.

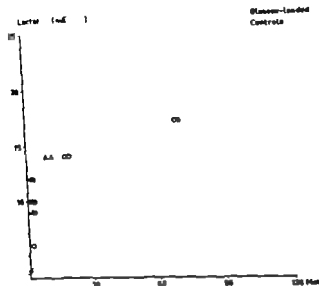


Fig. 3. Blood lactate during anoxia.

**pH** (Fig. 1) Blood pH fell successively in both groups. The values in the glucose-loaded foetuses tended to be somewhat lower than in the controls at the end of anoxia.

**K** (Fig. 2) A rise in the plasma potassium occurred in both groups, and was significantly higher in the control foetuses than in the glucose-loaded foetuses in the intervals 0-10 min ( $P < 0.01$ ) 11-30 min ( $P < 0.01$ ) and 31-50 min ( $P < 0.01$ ).

**Lactate** (Fig. 3) The lactate concentration increased greatly in both groups. The main increase occurred during the first 50 minutes. There was a significant difference between the mean values obtained at 0-10 min and 50-70 min ( $P < 0.001$ ).

**Na and Cl** (Fig. 4) The levels of sodium and chloride remained fairly stable during the time of the experiment.

**PO** (Fig. 5) During anoxia the concentration of inorganic phosphorus in plasma rose successively. The increase was significant ( $P < 0.001$ ) with no difference between the groups.

**Ca** (Fig. 6) The plasma calcium remained fairly unchanged in both groups during anoxia.

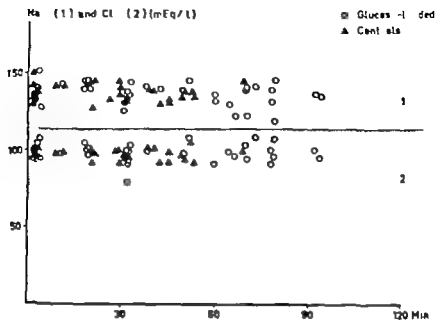


Fig. 4 Plasma sodium and chloride during anoxia.

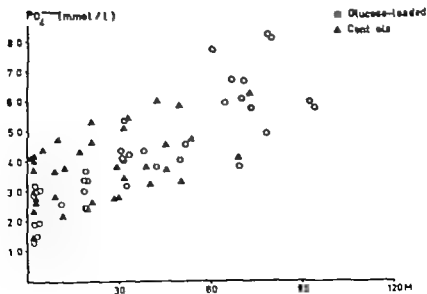


Fig. 5. Inorganic phosphorus in plasma during anoxia

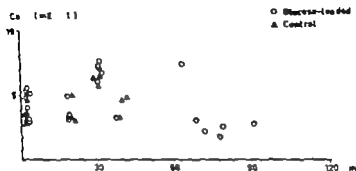


Fig. 6. Plasma calcium during anoxia.

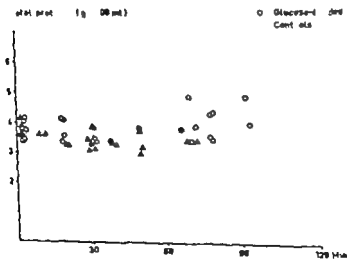


Fig. 7. Plasma protein during anoxia.

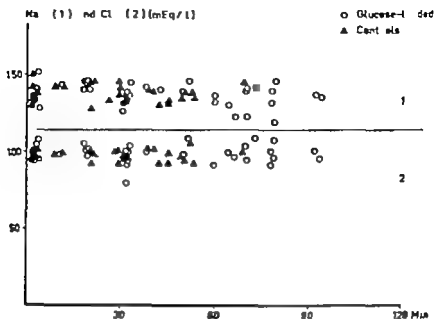


Fig 4 Plasma sodium and chloride during anoxia.

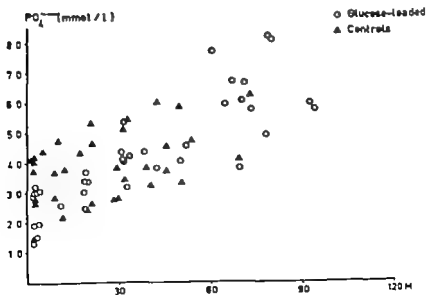


Fig 5 Inorganic phosphorus in plasma during anoxia.

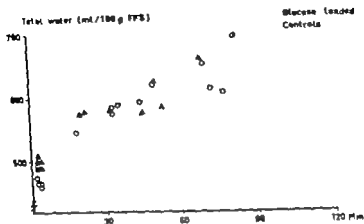


Fig. 9 Total water in heart muscle during anoxia (FFS = fat-free solids).

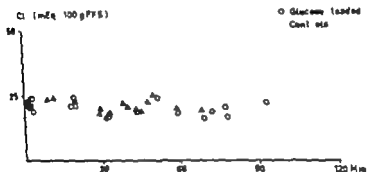


Fig. 10 Chloride content of the heart muscle during anoxia.

**Excess water ( $H_2O_e$ )** (Fig. 11) This showed a marked rise according to the same pattern as the increase in total water

**K** (Fig. 12) and **K<sub>e</sub>** During anoxia, a marked increase took place in both total K and excess K (**K<sub>e</sub>**). The rise in potassium content was most rapid during the first 30 minutes. It showed the same course in both groups, i.e. no significant difference was present when foetuses from both groups were in-

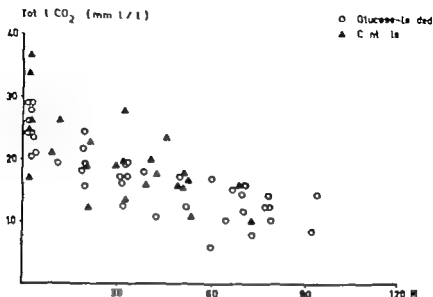


Fig. 8 Foetal carbon dioxide in plasma.

*Total protein* (Fig. 7) The concentration of total protein remained fairly stable and of the same magnitude in both groups.

*Total CO<sub>2</sub>* (Fig. 8) The total CO<sub>2</sub> fell successively during anoxia in both groups.

#### *Muscle electrolytes* (Table I)

The mean and range of H<sub>2</sub>O, Cl<sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>O, K<sup>+</sup>, K<sup>+</sup>, lactate, Na<sup>+</sup>, Na<sup>+</sup>, P, the K<sup>+</sup>/P ratio and Mg<sup>2+</sup> are given in Table I. The individual values are given in Figs. 9–15.

*H<sub>2</sub>O* (Fig. 9) The water content was initially almost significantly higher in the control group than in the experimental one ( $P < 0.05$ ). The content rose greatly in both groups. The main increase took place during the first 30–40 min. After 30 minutes no significant difference is present between the groups.

*Cl<sup>-</sup>* (Fig. 10) No significant change in the total chloride content occurred in either group.

*H<sub>2</sub>O<sub>2</sub>* In similarity to the total chloride the chloride space underwent no significant change in either group.

K	range	47.5-53.2	45.6-47.2	50.2-61.5	53.6-61.3	50.9-71.7	60.6-69.2	61.0-67.4	63.5-77.4	68.7-85.9
mEq/100 g		49.5	46.7	59.8	8.0	65.7	66.4	64.7	70.4	77.7
ITS		5	5	5	3	7	6	4	3	4
$n_2$		5	5	3	3	5	5	4	2	4
K <sub>1</sub>	range	44.7-46.8	46.3-52.0	51.7-56.7	51.2-60.1	57.7-69.1	58.6-67.6	60.2-64.4	60.2-76.3	66.0-82.1
mEq/100 g		45.7	49.4	54.3	46.6	63.0	64.8	62.5	66.9	74.5
ITS		5	5	5	3	7	6	4	3	4
$n_2$		5	5	3	3	5	5	3	2	4
Laurel	range	8.1-22.4	6.9-18.6	19.0-38.9	25.4-28.1	27.5-54.3	36.3-48.8	39.4-56.3	43.0-64.0	48.0-48.7
moist/100 g		14.2	13.0	27.3	26.8	44.1	42.2	47.9	56.2	60.7
dry weight		4	7	5	4	7	9	6	7	7
$n_2$		4	7	4	2	4	7	2	5	6
N	range	23.9-32.6	23.3-28.0	24.4-29.5	24.8-32.2	27.1-32.9	23.8-28.2	27.9-35.3	25.2-29.0	24.2-29.4
mEq/100 g		27.9	25.5	27.4	30.0	29.6	26.6	31.2	27.1	26.1
ITS		5	5	5	3	7	6	4	4	4
$n_2$		5	5	3	3	5	5	3	2	4
Na	range	-16.21	3.4-0.9	1.1-1.9	-0.7-0.4	1.1-6.0	-0.3-5.9	1.9-6.5	4.4-4.6	0.8-5.1
mEq/100 g		13	1.0	0.3	-0.1	3.9	2.4	3.5	4.5	2.6
$n_2$		5	5	5	3	7	5	4	3	4
$n_2$		5	5	3	3	5	5	3	2	4
P	range	41.6-46.2	41.7-46.2	41.9-47.1	42.1-45.6	42.5-47.0	42.1-47.3	45.0-49.4	43.0-45.9	44.5-49.5
moist/100 g		44.4	43.5	44.2	44.3	44.7	44.5	47.2	44.5	46.7
ITS		5	5	5	3	7	6	4	3	4
$n_2$		5	5	3	3	5	5	3	2	4



Table I Water and Electrolytes in Heart Muscle

Oxidation (min)	0-10		11-30		31-50		51-70		71-90		91-110	
	Contr	Gluc	Contr	Gluc	Contr	Gluc	Contr	Gluc	Contr	Gluc	Contr	Gluc
range	479-509	459-494	567-589	531-560	580-703	573-625	583-641	567-646	605-698			
$\bar{x}$	496	472	578	546	629	592	615	607	652			667
$n_1$	5	5	4	3	6	6	4	3	4			1
$n_2$	5	5	3	3	5	5	3	2	4			
range	20.9-23.4	18.9-22.8	19.2-21.7	20.3-24.1	18.6-22.4	16.1-19.9	19.9-22.2	17.4-30.0	16.5-19.9			
$\bar{x}$	21.8	21.1	20.6	21.6	20.5	17.8	20.8	23.7	18.7			21.8
$n_1$	5	5	5	3	7	6	4	3	4			1
$n_2$	5	5	3	3	5	5	3	2	4			
range	197-213	185-201	100-208	177-219	186-211	146-207	186-213	153-171	158-183			
$\bar{x}$	204	191	197	196	192	171	201	162	172			212
$n_1$	5	5	5	3	7	6	4	3	4			1
$n_2$	5	5	3	3	5	5	3	2	4			
range	276-318	263-314	385-388	304-370	395-487	377-487	391-425	410-481	417-535			
$\bar{x}$	295	280	387	346	432	419	414	446	487			434
$n_1$	5	5	5	3	7	6	4	3	4			1
$n_2$	5	5	3	3	5	5	3	2	4			
range	45.5-53.8	44.4-51.2	52.1-62.6	51.7-62.7	62.0-72.9	63.3-71.8	54.9-74.5	63.6-77.0	68.7-80.4			
$\bar{x}$	50.3	48.2	58.5	56.7	65.9	67.4	68.0	70.6	75.6			80.4
$n_1$	6	9	8	10	11	11	6	3	7			1
$n_2$	6	9	5	7	6	9	4	2	7			

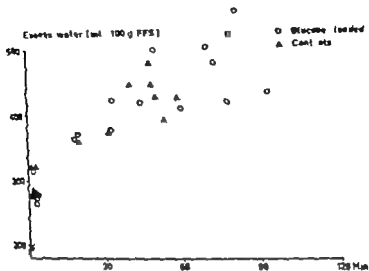


Fig. 11 Excess water in heart muscle during anoxia.

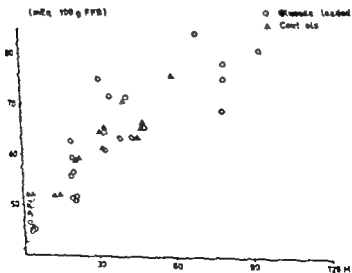


Fig. 12 Potassium in heart muscle during anoxia.

Table 1 (continued)

Foetuses	0-10	11-30		31-50		51-70		71-90		91-110	
		Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.
K/P ratio	range	11-12	10-12	13-14	12-15	14-16	13-16	13-15	14-18	13-18	
	$\bar{x}$	11	11	14	13	15	15	14	16	16	17
	$n_1$	5	5	5	3	7	6	4	3	4	1
	$n_2$	5	5	3	3	5	5	3	2	4	4
Mg mEq/100 g TFS	range	91-101	82-106	77-97	80-103	79-105	75-98	70-104	71-98		
	$\bar{x}$	96	96	90	93	95	92	89	89	92	93
	$n_1$	7	9	8	10	12	11	7	3	7	1
	$n_2$	7	9	5	7	7	9	5	2	7	7

Contr = control foetuses Gluc = foetuses of glucose loaded mothers  $\bar{x}$  = mean value  $n_1$  = number of foetuses  $n_2$  = number of litters  
K = determined by neutron activation analysis

When during one period determinations from several foetuses from the same litter had been made ( $n_1 > n_2$ ) a mean value for the litter was calculated. Thus within every cell in the table there are  $n_2$  observations, which can be regarded as independent. The mean and the range of the group were calculated from these  $n_2$  values

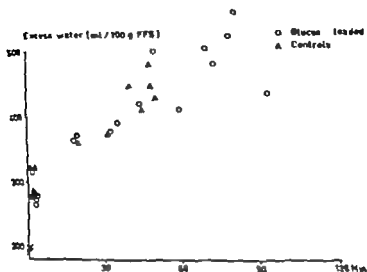


Fig 11 Excess water in heart muscle during asoxia.

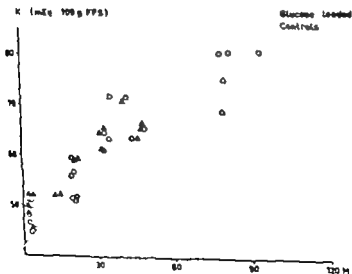


Fig 12 Potassium in heart muscle during asoxia.

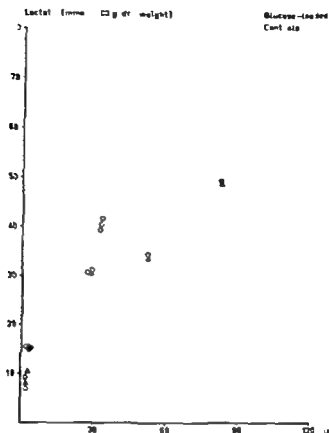


Fig. 13 Lactate in heart muscle during anoxia.

investigated concurrently. In the glucose-loaded group—whose heart activity had a longer duration—the rise in  $K^+$  was much greater at the end of the period of anoxia than that in the control group (an increase of 32 mEq and 18 mEq/100 g FFS respectively). The increase was highly significant in both groups ( $P < 0.001$ ). The mean value for the last animal of each litter was significantly higher in the experimental group than in the control group ( $79.9 \pm 1.6$  and  $67.8 \pm 2.0$  mean  $\pm$  S.E. respectively,  $P < 0.01$ ).

**Lactate** (Fig. 13) The concentration rose markedly during the whole period of anoxia. No significant difference was present when foetuses from both groups were compared at the same time interval. At the end of anoxia the increase was greater in the experimental group than in the control group ( $49.3 \pm 3.3$  and

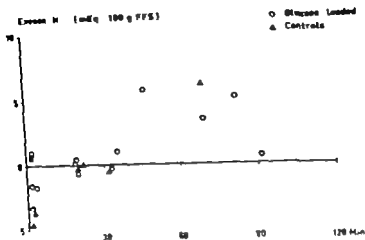


Fig 14 Excess sodium in heart muscle during anoxia

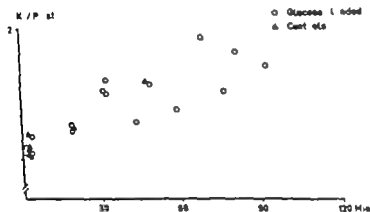


Fig 15 K/P ratio in heart muscle during anoxia

$33.4 \pm 5.4$  mean  $\pm$  S.E. respectively) In the glucose loaded group the mean value at the end of anoxia for the last animal of each litter was almost significantly higher in the experimental group than in the control group (62.3 and 47.8 respectively  $P < 0.05$ )

$Na$  and  $Na$  (Fig 14) The  $Na$  content did not change significantly whereas excess  $Na$  increased significantly in both groups ( $P < 0.01$ ) A tendency to a decrease in  $Na$  did, how

ever occur at the end of the period of anoxia (after about 50 minutes) in the glucose-loaded fetuses

*Phosphorus* No significant change occurred during anoxia in either group

*K / P ratio* (Fig. 15) The K / P ratio rose considerably in both groups. At the end of the period the rise was much greater in the glucose-loaded group than in the untreated one. The difference is significant ( $P < 0.001$ )

*Mg* The content did not change significantly during the period of anoxia. No difference was present between the groups.

### Discussion

During anoxia a considerable increase took place in the plasma concentration of potassium, lactate and phosphate in both the glucose-loaded fetuses and the controls while the blood pH fell. On the other hand no definite changes occurred in the calcium, sodium, chloride and protein concentrations. Similar results with respect to potassium, lactate and pH in fetuses under anoxia have been reported previously (Colldahl 1947, Salmi 1954, Brandt *et al.* 1958, Thalme 1967)

The rise in potassium concentration of the plasma during the first 50 minutes of anoxia was significantly lower in the experimental group.

In the present series the total  $\text{CO}_2$  was also determined. It decreased during anoxia concurrently with a rise in lactate and a fall in pH.

In the heart muscle a pronounced increase was noted in the total water, total potassium and lactate in relation to dry weight. The total quantity of chloride, sodium and phosphorus had not undergone any definite change; nor had the chloride space (i.e. the distribution volume of chloride). On the other hand excess sodium—i.e. sodium outside the chloride space—which was initially negative in most fetuses, rose during the period of anoxia.

If the pronounced increase in total water had to a great extent consisted of an increase in extracellular fluid, both the chloride space and the sodium space should have shown a considerable percentage increase. Since this did not occur, our results indicate

that the increase in water consisted mainly of an increase in intracellular water. This is in accordance with findings in skeletal muscle of working rats in hypoxia (Rooth 1966). The increase took place chiefly during the first 40 minutes. The lower heart water content in the experimental group as compared to the control group at the beginning of anoxia may have been due to the initial hyperglycaemia (Gelli *et al.* 1968) which has an osmotic effect on the cells.

The potassium content of the heart muscle of anoxic foetuses is of great interest. In relation to the dry fat free weight, the potassium content increased by no less than about 30 per cent in the untreated group and by about 60 per cent in the glucose-loaded one. This difference was significant. The course of the potassium increase was the same in both series. However higher values were reached by the glucose loaded foetuses which seems to be related to their longer heart activity. It was previously shown that the heart muscle had a higher initial glycogen content in the foetuses of glucose-loaded mothers, and that their heart activity had a longer duration under anoxia (Gelli *et al.* 1966 and 1968).

From the point of view of electroneutrality it is inconceivable that potassium can be accumulated to the degree which occurred in our experiments without a concurrent increase in the content of intracellular anions.

In anaerobic metabolism, lactic acid is formed, *i.e.* lactate ions + hydrogen ions. Our results demonstrate that a considerable amount of lactate accumulates in the heart, and that the increase in lactate is greater than the increase in potassium. The possibility exists both that hydrogen ions are buffered intracellularly and that they leave the heart and are buffered in extracellular fluid or in other organs.

Eckel, Borschner and Wood (1959) attempted to determine the buffering capacity of skeletal muscle by titration of muscle homogenates at 0 °C. On titration in the pH interval 6–7 they found a buffering capacity of about 16 mEq/l H<sup>+</sup> per pH unit per 100 g FFS. It is possible that the buffering capacity of foetal heart muscle is much greater since its phosphate content is far higher than that of the skeletal muscle of adult animals.



On the other hand, a considerable amount of hydrogen ions must necessarily have left the heart muscle cells to permit the great  $K^+$  uptake to occur. The falling pH and total  $CO_2$  of the extracellular fluid suggest that hydrogen ions leave the cells. The quantitative role played by the heart in this respect cannot, however be established even if it can be envisaged to be considerable since the heart is the only working organ. In adult man the potassium/phosphorus ratio in skeletal muscle is relatively constant, and cannot be increased to any appreciable degree despite the presence of hyperkalaemia (Bergström 1962). The marked rise in the potassium/phosphorus ratio above the initial value in the anoxic heart shows that the conditions are not similar to those in the skeletal muscle. This confirms that extra anion must have been added to permit the accumulation of potassium.

As previously mentioned excess sodium rose during the period of anoxia. This can be interpreted either by the occurrence of an outflow of chloride from the cells without a concomitant release of sodium, or by sodium entering the cells or possibly by both events taking place concurrently.

Whatever the events may be this nevertheless implies that the intracellular cation-anion balance is changed in such a way that sodium as well as an extra cation can correspond to a given amount of intracellular lactate ions.

It is known from studies in experimental animals and in man that a reciprocal relation is generally present between intracellular sodium and potassium. Such a reciprocal relation does not, however seem to exist in the anoxic foetal heart. Instead both potassium and excess sodium increase during anoxia.

The amount of potassium is well correlated to the total water content (Fig. 16). This indicates that the intracellular increase in water in the heart is caused by an increase in the number of osmotically active particles. I.e. on an accumulation of potassium and lactate water is drawn into the cardiac cells which swell. This also implies that—although the quantity of potassium per FFS increases—the intracellular potassium concentration does not increase or only inappreciably. Since the extracellular potassium concentration increases greatly this denotes that the  $K^+ / K_e$  ratio decreases. If potassium is distributed according to the Nernst

equation (Conway 1957) this would imply that the resting membrane potential decreases continuously in the heart during anoxia.

One of the basic aims of this study was to ascertain whether the prolongation of heart activity in the foetuses of glucose loaded and of untreated mothers could in fact, be explained by some other factor than the increased glycogen content of the heart in the former group e.g. differences with respect to the electrolyte metabolism. When foetuses from the respective groups were compared with regard to electrolyte content of muscle after an equally long period of anoxia there was however no definite difference between the groups with respect to any electrolyte. The foetuses of glucose-loaded mothers—which generally had a longer recordable heart activity—consequently continued to accumulate potassium. Their final values were thus significantly higher at the end of anoxia than those in the control foetuses.

We have interpreted this to mean that the potassium accumulation as such is not a limiting factor but that it is secondary to the accumulated amount of lactate which, in turn is dependent on the initial amount of glycogen. This was greater in the glucose-loaded group (Gelli *et al.* 1966 and 1968) which also had the longest recordable heart activity and, therefore also the greatest glycogen utilization.

The mechanism of the ion and water accumulation in the anoxic foetal heart is unknown. It is nevertheless evident that an active transport is required for this purpose either of potassium or hydrogen ions or through a coupled ion-transport mechanism. The energy for this transport is obtained through glycolysis, since the animal is anoxic. Sodium presumably increases intracellularly. This increase may depend either on an increased sodium permeability i.e. a passive entry of sodium into the cell, or on a decreased active transport of sodium from the cell. Rooth (1966) has recently postulated an active cation pump in skeletal muscle which in hypoxia will pump  $H^+$  out from the cells with a concurrent decrease in  $Na^+$  elimination. The ability to extrude  $H^+$  from the anoxic cells may be one of the vital functions which cease when the energy supply is insufficient because of glycogen depletion.

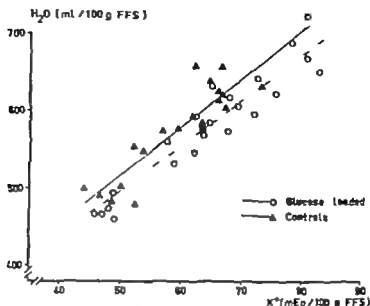


Fig. 16 Potassium correlated to the total water content in heart muscle.

### SUMMARY

The electrolyte conditions in the heart muscle and in plasma were studied during anoxia in glucose-loaded and in control foetuses. The changes in plasma electrolytes showed the pattern already known i.e. a great increase in lactate and a fall in pH and total CO<sub>2</sub>. Initially the glucose-loaded group had lower plasma potassium values than the control group.

The water content, potassium and lactate in the heart muscle increased markedly during anoxia. The glucose-loaded group reached higher final values of K<sup>+</sup> and lactate. Chloride, total sodium, phosphorus and magnesium showed no significant changes, whereas excess Na<sup>+</sup> increased significantly in both groups. The results indicate that during anoxia an accumulation of lactate takes place intracellularly in the heart muscle and that K<sup>+</sup> and to some extent Na<sup>+</sup> enter the cells with a concurrent release of H<sup>+</sup> from them. Water is drawn into the cells through the osmotic effect exerted by the accumulated ions.

### Acknowledgments

This paper was supported by grants from Professor Erik Ahlström's fond för obstetrisk-gynekologisk forskning 1968 and Expressens Prenatalforskningsfond 1967.

## REFERENCES

- Berrier S. B. and Simonsson W. H., *J. biol. Chem.* 138 535 1941
- Bergström J. *Scand. J. clin. Lab. Invest.* 14 suppl. 68 1962
- Bromide V. Bergström J. and Hultman E., *T. be published* 1968
- Bresch I. K., Harrod, H. S. J. and Cooke R. E. *Amer J Physiol.* 193 263, 1958
- Callins, E., Taylor J. M. and Hastings, A. B. *Amer J Physiol.* 177 211 1954
- Call Dahl H. *Acta nord. scand.* 128 417 1947
- Conney E. J. *Physiol. Rev.* 37 84 1957
- Eckel R. E., Borckner A. W. and Wood, D. H. *Amer J Physiol.* 196 811 1959
- Eurwonen A. J. Merckxde L. B. and Peters, J. P. *J. biol. Chem.* 116 33 1938
- Gelli M. G. Enghörning, G. Hultman E. and Bergström J. *Proceedings of symposium on Problems of Foetal Distress, Siena, Italy September 1966*
- Gelli M. G. Enghörning, G. Hultman E. and Bergström J. *T. be published in Acta paediat. (Uppsala)* 1968
- Gelli M. G. and Gynälä F. *Acta obstet. gynec. scand.* 48 56 1959 1968
- Rome, G. Kahn D. S. and Cheppel, C. J. *Electrolytes and Cardiovascular Diseases* ed. by E. Bajusz, pp. 181 191 S. Karger Basel/New York 1963
- Roth G. *Clin. Sci.* 30 417 1966
- Salm I. *Ann. Paediat. Fenn.* 3 suppl. 2, 1954
- Sam M. C. *Clin. Chem.* 3 406 1957
- Deutsches Med. J.* 13 811 1962
- Scholz R., Schmeitz H. Bäcker Th. and Lampen J. O. *Biochem. Z.* 331 71 1959
- Selje H. and Gabbiani G. *Electrolytes and Cardiovascular Diseases*, ed. by E. Bajusz, pp. 135-160 S. Karger Basel/New York 1963
- Thabue B. *Acta paediat. (Uppsala)* 51 649 1962
- Acta med. scand.* 174 suppl. 403 1963
- Acta obstet. gynec. scand.* 43 78 1964
- Acta obstet. gynec. scand.* 45 suppl. 8 1967

Received on Feb. 22, 1968

## EFFECT OF GLUCOSE INFUSION IN THE MOTHER BEFORE DELIVERY ON THE ECG OF RABBIT FOETUSES UNDER ANOXIA

BY

M. G. GELLI AND F. GYULAI

In a previous study the effect was investigated of glucose infusion in the mother before delivery on the heart activity of rabbit foetuses under anoxia (Gelli *et al.* 1966 and 1968a). It was found that asystole appeared much later in the glucose-treated group than in the controls. An account is given in this paper of the way in which the various ECG parameters underwent changes during anoxia in both groups.

### *Material and Methods*

Twenty six rabbit foetuses removed by laparotomy on the 29th day of pregnancy were investigated. 13 were used as controls and the same number as experimental animals whose mother had been given a glucose infusion before delivery. For the technique reference is made to earlier reports (Gelli *et al.* 1966 and 1968a).

The standard ECG in lead I was recorded with an Elema Mingo-graph 12 B from 5-6 minutes after the onset of anoxia and during the whole experiment, i.e. generally as long as any heart activity was present. In some cases however the recording was discontinued after the cessation of ventricular activity. The paper speed was 25 mm/second during continuous recording but interesting sections were recorded at a rate of 100 mm/second.

The ECG was analyzed at a magnification of 7 X and with an accuracy of  $\pm 0.1$  mm, which corresponds to 0.001 second (at the

## Control

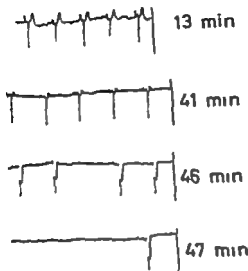


Fig. 1 Control.

13 min Normal ECG 41 min Slight S-T depression. 46 min Extreme S-T depression 47 min Complete A-V block ventricular rate <10/min.

last-mentioned paper speed) Measurements were made routinely every 8-20 minutes, as well as immediately before any major changes (e.g. appearance of S-T depression or A-V block)

## Results

## Controls (Fig. 1 Tables I and II)

Initially there was sinus rhythm, with a mean rate of 85/min. The P waves were positive and the mean P-Q time 60 msec with a relatively small range (40-70 msec). The S-T segment was isoelectric in all but two cases in which it was depressed. The T waves were positive. The Q-T time was a mean 185 msec.

Table 1 ECG Parameters after Varying Periods of Anoxia.  
 Contr = Controls. Gluc = Glucose loaded Foetuses

Anoxia (min)	6-10		30-40		41-62		63-87		88-	
Foetuses	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.
P-Q (msec)										
mean	58	69	73	73	102	136	177	250		
range	40-70	40-160	60-100	50-120	80-140	45-200	175-180	150-360		
No	13	13	12	13	9	11	2	4		
Heart rate (beats/min)										
mean	85	70	68	61	53	36	33	25		
range	60-120	40-93	10-108	19-90	10-115	10-52	20-46	15-52		
Q-T (msec)										
mean	185	192	153	180	144	163	146	180	(101-102 min)	
range	150-240	160-220	130-200	140-280	100-170	120-200	120-160	120-260	200	
No	13	13	12	13	9	11	2	7	160-240	
Heart rate (beats/min)									2	
mean	85	70	68	55	53	33	33	ca 21		
range	60-120	40-93	10-108	19-90	10-115	10-52	20-46	<10-50	<10	

During the first 40 minutes, the ECG showed comparatively few changes. The rate fell to about 68/min the conduction time increased, and the Q-T time decreased slightly. At the end of this period, depression of the S-T segment appeared, and increased markedly during the next few minutes after on an average 49 minutes anoxia, the T waves became negative.

After 52 minutes anoxia, an A-V block appeared with an extremely low ventricular rate (usually  $\leq 10/\text{min}$ ) but no concurrent change in atrial rate. From this time onwards, bursts of ventricular extrasystoles frequently occurred.

The conduction time first increased slowly then successively more and shortly before the appearance of A-V block was a mean 100 msec. The heart rate had, however, fallen concurrently to slightly over 50/min. The Q-T time, on the other hand, fell continuously despite a coincident decrease in heart rate, and during the period 41-62 minutes anoxia was considerably lower than the initial value i.e. around 140 msec.

The mean value of the duration of heart activity in this group was 64 minutes.

#### Experimental animals (Fig. 2, Tables I and II)

At the beginning of the recording, there was sinus rhythm the mean rate being 70/min. The P waves were positive and the P-Q time about 70 msec. The S-T segment was isoelectric, except in one case in which it was depressed. The T waves were positive. The Q-T time was about 190 msec.

During the next 40 minutes the heart rate fell to a mean 61/min whereas the P-Q time and Q-T time were largely unchanged. The conduction time showed a marked, successive increase and in 4 cases in which it could be measured in the period 63-84 minutes anoxia it was a mean 250 msec. Concurrently the heart rate was about 25/min. In the foetuses of the glucose-loaded mothers no major changes occurred in the Q-T time during anoxia. Since there was a concomitant marked decrease in the heart rate this must imply a relative shortening of the Q-T time. In these foetuses, the mean total duration of heart activity was 82 minutes.

After the first 40 minutes these 13 ECG's could be divided into



## Glucose-infused

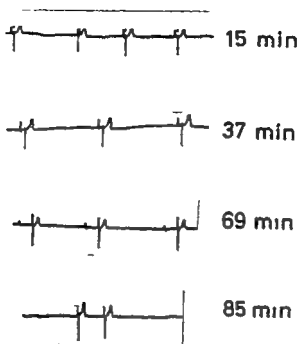


Fig. 2. Experimental foetus

15 min: Normal ECG 37 min: Normal ECG S-T segment isoelectric. T wave positive 69 min: Prolonged P-Q time. Mainly unchanged ECG 85 min: Complete A-V block. Ventricular rate ~10/min. No S-T depression. T wave positive.

two groups. In 7 cases an S-T depression appeared after about 43 minutes anoxia. It subsequently increased and the T waves became negative after a few minutes *i.e.* at about the same time as in the controls. In this group the heart activity persisted for a mean 71 minutes. In the other 6 cases no S-T depression occurred. The T waves remained positive for practically the whole time (87.5 min). The heart activity ceased after a mean 94 minutes anoxia.

## Comments

Infusion of glucose in the mother animal before delivery resulted in higher glycogen content of the foetal heart muscle of about

Table II. Time of Appearance During Anoxia of S-T and T Changes as well of Atrio-Ventricular Dissociation and Total Duration of Ventricular Activity Respectively

Contr. Controls. Gluc. = Foetuses of Glucose-loaded Mothers

Foetuses	Contr.	Gluc.
Negative T waves (min anoxia)		
mean	49	67
range	19-74	29-104
S-T depression (min anoxia)		
mean	39	43*
range	10-68	10-75
A V block (min anoxia)		
mean	52	64
range	21-79	29-97
Ventricular activity (min anoxia)		
mean	64	62
range	45-87	54-116

7 cases (no S-T depression in 6 cases)

10 cases (no A V block in 3 cases)

40 per cent. During anoxia this difference from the controls could be demonstrated during the whole period of heart activity. In the foetuses of glucose-loaded mothers, the heart activity had a longer duration, but the muscle glycogen—after a continuous decrease in both groups—reached a level of about 0.4 g/100 g wet weight when the heart activity ceased (Gelli et al. 1968 a).

Although the ultrastructure of the cardiac muscle cells showed the same pathological changes in both groups, they appeared later in the glucose-loaded animals than in the controls (Gelli et al. 1968 b).

Initially the electrolyte values appeared similar and the intracellular potassium increased with time at the same rate. In the final phase this resulted in high potassium values in the foetuses of glucose-treated mothers since their heart activity continued for

## Glucose-infused

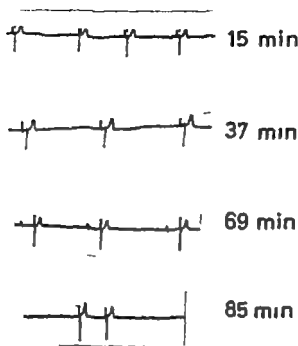


Fig 2 Experimental foetus.

15 min Normal ECG 37 min Normal ECG S-T segment isoelectric. T wave positive 69 min: Prolonged P-Q time Mainly unchanged ECG 85 min Complete A-V block Ventricular rate ~10/min. No S-T depression. T wave positive

two groups In 7 cases an S-T depression appeared after about 43 minutes anoxia it subsequently increased, and the T waves became negative after a few minutes *i.e.* at about the same time as in the controls In this group the heart activity persisted for a mean 71 minutes. In the other 6 cases no S-T depression occurred. The T waves remained positive for practically the whole time (87.5 min) The heart activity ceased after a mean 94 minutes anoxia

## Comments

Infusion of glucose in the mother animal before delivery resulted in higher glycogen content of the foetal heart muscle of about

Table II. Time of Appearance During Anoxia of S-T and T Changes as well as of Atrio-Ventricular Dissociation and Total Duration of Ventricular Activity Respectively

Contr Controls Gluc. = Foetuses of Glucose-loaded Mothers.

Foetuses	Contr	Gluc.
Negative T waves (min anoxia)		
mean	49	67
range	19-74	29-104
S-T depression (min anoxia)		
mean	39	43*
range	10-68	10-75
A-V block (min anoxia)		
mean	52	64
range	27-79	29-97
Ventricular activity (min anoxia)		
mean	64	82
range	45-87	54-118

7 cases (no S-T depression in 6 cases).

10 cases (no A-V block in 3 cases).

40 per cent. During anoxia this difference from the controls could be demonstrated during the whole period of heart activity. In the foetuses of glucose-loaded mothers the heart activity had a longer duration, but the muscle glycogen—after a continuous decrease in both groups—reached a level of about 0.4 g/100 g wet weight when the heart activity ceased (Gollt *et al.* 1968 a).

Although the ultrastructure of the cardiac muscle cells showed the same pathological changes in both groups, they appeared later in the glucose-loaded animals than in the controls (Gollt *et al.* 1968 b).

Initially the electrolyte values appeared similar and the intracellular potassium increased with time at the same rate. In the final phase this resulted in higher potassium values in the foetuses of glucose-treated mothers, since their heart activity continued for

## Glucose-infused

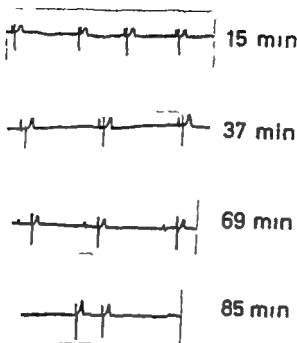


Fig 2 Experimental foetus.

15 min Normal ECG 37 min Normal ECG S-T segment isoelectric. T wave positive 69 min Prolonged P-Q time. Mainly unchanged ECG 85 min Complete A-V block. Ventricular rate ~10/min. No S-T depression. T wave positive.

two groups In 7 cases an S-T depression appeared after about 43 minutes anoxia it subsequently increased, and the T waves became negative after a few minutes *i.e.* at about the same time as in the controls. In this group the heart activity persisted for a mean 71 minutes In the other 6 cases no S-T depression occurred The T waves remained positive for practically the whole time (87.5 min) The heart activity ceased after a mean 94 minutes anoxia

### Comments

Infusion of glucose in the mother animal before delivery resulted in higher glycogen content of the foetal heart muscle of about

during the later part of anoxia (Gelli *et al.* 1968 c) may also contribute to higher and, therefore more distinct T waves in the latter cases. The differences in P-Q and Q-T times were, however consistently small, and they will be studied in greater detail.

### SUMMARY

It has previously been shown that rabbit foetuses whose mother had received a glucose infusion before delivery had a longer duration of heart activity under anoxia than the controls. The present study comprises an analysis of the ECG's during the whole period of anoxia in both groups. The mean duration of heart activity was 64 minutes in the controls, and 82 minutes in the glucose-loaded foetuses.

Depression of the S-T segment occurred soon after 40 minutes anoxia in the controls. In 50 per cent of the glucose-loaded foetuses no S-T depression occurred, and in the other 50 per cent it appeared 13 minutes later than in the controls.

The conditions were approximately the same with respect to the T waves. An A-V block appeared in the controls after about 52 minutes anoxia. No block occurred in 3 of the foetuses of glucose-loaded mothers, and in the rest it appeared 12 minutes later than in the controls.

### REFERENCES

- Gelli M G, Enghörning G, Hultman E. and Bergström J. Proceedings of Symposium on Problems of Fetal Distress, Siena, Italy September 1968  
To be published in *Acta Paediat. Scand.* 1968 a  
Gelli M C, Eriksson J L E. and Enghörning, C. To be published in *Acta Paediat. Scand.* 1968 b  
Gelli M C, Bergström J, Hultman E. and Thalmé B. *Acta Obstet. Gynec. Scand.* 43: 34 1959

Received on Feb. 22 1968

a longer time. The extracellular pH behaved analogously and therefore reached lower values in this group (Gelli *et al.* 1968 c).

During anoxia pathological changes in the ECG took place successively but not at the same rate nor to the same extent in the glucose loaded foetuses as in the controls.

The most striking difference was with respect to the S-T segment. Depression of this segment is known to be a sign of myocardial ischaemia which may be due either to generalized hypoxia or to a localized disturbance in the oxygen supply to the myocardial cells.

The total duration of heart activity in the 6 glucose-loaded cases with no S-T depression was a mean 94 minutes (range 72-118 min) as compared to a mean 71 minutes (range 49-99 min) in the remaining 7 foetuses of glucose-treated mothers. The corresponding duration in the controls was 64 minutes (range 45-87 min). Moreover in the aforementioned 6 cases the T wave was positive for practically the whole time (87.5 min) whereas in the other 7 cases it became negative after about 48 minutes anoxia. Thus normal S-T segments and T waves—reflecting better myocardial function during heart activity under anoxia—were demonstrated in about half the foetuses of glucose-loaded mothers. More detailed ECG studies with several leads are required to establish the cause of the differences observed.

An A-V block with low ventricular rate is an expression of poor myocardial function. In all controls an A-V block appeared after a mean 52 minutes anoxia whereas no block occurred in 3 experimental animals and in the rest of them the onset was later i.e. after about 64 minutes.

The conduction time (P-Q time) increased during anoxia in both groups first slowly and then increasingly rapidly. The fact that the foetuses of glucose-loaded mothers finally reached higher values can be explained by the longer duration of their heart activity. In addition their slightly lower heart rate may have been a contributory factor. The somewhat longer Q-T time in the glucose-loaded foetuses may have been due to the depression of the S-T segment making the T waves isoelectric or negative to a greater extent in the controls than in the foetuses of glucose-treated mothers. The high potassium content of the heart muscle

during the later part of anoxia (Gelli et al 1968 c) may also contribute to higher and, therefore more distinct T waves in the latter cases. The differences in P-Q and Q-T times were however consistently small, and they will be studied in greater detail.

### SUMMARY

It has previously been shown that rabbit foetuses whose mother had received a glucose infusion before delivery had a longer duration of heart activity under anoxia than the controls. The present study comprises an analysis of the ECG's during the whole period of anoxia in both groups. The mean duration of heart activity was 64 minutes in the controls, and 82 minutes in the glucose-loaded foetuses.

Depression of the S-T segment occurred soon after 40 minutes anoxia in the controls. In 50 per cent of the glucose-loaded foetuses no S-T depression occurred, and in the other 50 per cent it appeared 13 minutes later than in the controls.

The conditions were approximately the same with respect to the T waves. An A-V block appeared in the controls after about 52 minutes anoxia. No block occurred in 3 of the foetuses of glucose-loaded mothers and in the rest it appeared 12 minutes later than in the controls.

### REFERENCES

- Gelli M G, Euhörning, G, Hultman E. and Bergström J. Proceedings of Symposium on Problems of Fetal Distress, Sessa, Italy September 1965  
To be published in *Acta Paediatr. Scand.* 1968  
Gelli M G, Ericson J L E. and Euhörning, G. To be published in *Acta Paediatr. Scand.* 1968 b  
Gelli M G, Bergström J, Hultman E. and Thabae B. *Acta Obstet. Gynec. Scand.* 48: 34 1969

Received on Feb 22, 1968



*From the Institute of Medical Microbiology Department of Bacteriology (Prof Ö Ouchterlony) University of Göteborg, and the Department of Obstetrics and Gynaecology II (Prof P Bergman) Sahlgrenska sjukhuset Göteborg, Sweden*

## SPERM AGGLUTININS IN MALE BLOOD DONORS

BY

BO FJÄLLBRANT

### *Introduction*

Investigations of sperm antibodies in human males have been performed as studies of a few cases (*Wilson* 1954 1956) as determinations of the incidence in men with azoospermia (*Rümke* 1954) and obstructed vas deferens (*Phadke and Padukone* 1964) in a urological clientele (*Bandhauer* 1966) in sterile clienteles (*Fjällbrant* 1965 *Schwimmer et al* 1967) and as comparisons between the incidence in sterile and fertile groups (*Rumke and Hellings* 1959 *Fjällbrant* 1968). *Bandhauer* included in his investigation a control group of 50 patients who were treated for diseases unrelated to the genital tract in this control group he found no case of sperm agglutinin positivity (defined as a titre  $\geq 1/32$  with the method of *Kibrick*.)

Knowledge of the presence of sperm antibodies in healthy men is lacking but would be useful for interpreting results of studies in groups selected with regard to fertility or certain diseases. The aim of the present investigation was to determine the presence and titre of sperm agglutinins in a group of obviously healthy men not selected with regard to fertility and to compare the results with the incidence in fertile and sterile groups reported in an earlier investigation performed with the same technique (*Fjällbrant* 1968). Although no influence of the ABO blood groups on the results of the earlier investigation was found their possible influence was reconsidered.

### *Material and Methods*

The blood donors were 500 healthy men 20-45 years old (mean 30 years). Blood samples for testing with regard to sperm agglutinins were taken simultaneous to donation.

The ABO blood group was determined by testing their erythrocytes for antigens and their sera for antibodies.

The blood samples were centrifuged and the decanted sera kept at -30° C until examination.

**Spermatozoa.** Fresh ejaculates from one donor were used throughout the investigation. He belonged to blood group B and was a secretor of B-substance in his seminal plasma. Before use each ejaculate was controlled for volume, sperm density and percentage of motile spermatozoa. The means of these values for 11 ejaculates used in the sperm agglutinin determinations were as follows: volume, 5.5 ml (standard deviation, 0.21 ml) sperm density 105 million per ml (standard deviation, 14.7 million/ml) motile spermatozoa, 55 per cent (standard deviation, 5.7 per cent). The sperm motility was very rapid, and in other respects the samples were also normal.

The test for sperm agglutinins was performed with the method of Kibrick *et al.* (1952) slightly modified.

The serum was inactivated by heating at 56° C for 30 minutes and diluted 1:4 with Baker's solution (buffered glucose). A fresh ejaculate was diluted with Baker's solution to a sperm density of 40 million per ml. The diluted semen was mixed with an equal volume of 10 per cent gelatin in Baker's solution. An aliquot of 0.3 ml of the semen-gelatin mixture was mixed with 0.3 ml of the diluted serum, transferred to a small precipitation tube (5 × 65 mm) and incubated at +37° C for 2 hours. The presence of agglutinates was assessed macroscopically immediately after incubation. —For the titre determination the sperm agglutinin positive sera were serially diluted two-fold with Baker's solution. Each dilution was blended with the semen-gelatin mixture and incubated as described above. The highest dilution of serum before the blending with the semen-gelatin mixture that gave an obvious agglutination after incubation, was registered as the agglutinin titre.

The statistical analysis which involved comparisons between

Table I *Distribution of the Sera According to Sperm Agglutinin Titre*

Negative Sera	Sperm Agglutinin Titre (reciprocal)										Total
	4	8	16	32	64	128	256	512	1024	2048	
476	2	6	5	5	2	2	1	-	-	1	500

Table II *Distribution of Sperm Agglutinin Negative Sera and Sera with Low ( $\leq 1/32$ ) and High ( $\geq 1/64$ ) Titres*

	Total	Negative Sera	Positive Sera		Total
			Low Titres	High Titres	
Number	500	476	18	6	24
Per cent	100	95.2	3.6	1.2	4.8

Table III *The Distribution of the ABO Blood Groups in the Sperm Agglutinin Positive and Negative Groups, Per Cent Figures in ( )*

	A	O	B	AB	Total
Negative	213 (44.8)	188 (39.5)	53 (11.1)	22 (4.6)	476 (100.0)
Positive	11 (45.8)	11 (45.8)	2 (8.4)	- (0.0)	24 (100.0)
Total	224 (44.8)	199 (39.8)	55 (11.0)	22 (4.4)	500 (100.0)

distributions was performed with  $\chi^2$  tests. A difference was called significant when  $p < 0.05$ , highly significant when  $p < 0.01$  and almost significant when  $p < 0.1$ .

### Results

The distribution according to sperm agglutinin titre of the sera is given in Table I. There were 24 positive sera out of 500, an incidence of 4.8 per cent. The sera were classified into low ( $\leq 1/32$ ) and high ( $\geq 1/64$ ) titre categories. There were 18 (3.6

per cent) with low titres and 6 (1.2 per cent) with high titres, see Table II

The distribution of the ABO blood groups in the sperm agglutinin positive and negative men is given in Table III. Statistical analysis showed that there was no significant difference between the sperm agglutinin positive and negative groups in regard to the ABO blood group distribution ( $X^2 = 1.50$  d.f. = 3  $p > 0.1$ )

### *Discussion and Conclusions*

The test for sperm agglutinins used in this investigation was discussed in detail in an earlier paper (Fjällbrant 1968). In the investigation reported in that paper the ABO blood group and the presence and titre of sperm agglutinins was determined in 900 men, of which 400 lived in sterile marriages and 500 in fertile marriages. The ABO blood group distribution was almost the same in the sperm agglutinin positive and negative groups. Also in the present investigation it is noted that the ABO blood group distribution was very similar in the sperm agglutinin positive and negative groups of blood donors. Most notable is that two sperm agglutinin positive males belonged to the B group, although the semen used as antigen came from a man belonging to the B group. The similar blood group distribution in the sperm agglutinin positive and negative groups and the finding of men with sperm antibodies belonging to the B group would not have occurred if the ABO blood group agglutinins were a primary cause of agglutination in the sperm agglutination method used. It can be concluded that the ABO blood factors do not influence the results obtained with Kibrick's gelatin agglutination test.

In the aforementioned investigation the mean age of the men in the sterile group was 32 years (range 21–56 years) and in the fertile group 30 years (range 19–51 years) to be compared to a mean age of 30 years (range 20–45 years) of the blood donors. The present investigation was performed with the same technique and with use of the same semen donor. Since the groups were similar in regard to age and the investigation methods the same comparisons of the results of the investigations may be considered valid.

Table IV *Distribution (Per Cent) of Sperm Agglutinin Negative Sera and Sera with Low ( $\leq 1/32$ ) and High ( $\geq 1/64$ ) Titres in the Sterile Fertile and Blood Donor Groups Absolute Numbers in ( )*

	Total	Negative Sera	Positive Sera		Total
			Low Titres	High Titres	
Sterile	100.0(400)	93.2	2.5	4.3	6.8
Fertile	100.0(500)	97.4	1.8	0.8	2.6
Blood donors	100.0(500)	95.2	3.6	1.2	4.8

The incidence of sperm agglutinin positivity was 6.8 per cent in the sterile and 2.6 per cent in the fertile group. The incidence in the blood donors was 4.8 per cent or midway between the figures for the sterile and fertile groups.

In the earlier investigation it was found that the difference with regard to titre level between the sterile and the fertile groups was greatest if a limit was drawn between the titres  $1/32$  and  $1/64$ . Titres below this limit were called low and titres above high. The distribution of low and high titres in the sterile, fertile and blood donor groups is given in Table IV. The sterile group had the greatest incidence of high titres. The fertile group showed the smallest incidence both of low titres and of high titres. The blood donor group showed the greatest incidence of low titres; the incidence of high titres in the blood donor group was somewhat greater than in the fertile group but considerably smaller than that of the sterile group.

Statistical analysis showed that the differences between the sterile and fertile groups were significant except in regard to low titres. A statistical analysis of the difference between the blood donors on one hand and the sterile and fertile groups on the other gave the following results. The difference between the blood donors and the fertile group with regard to incidence of positive sera was almost significant ( $X^2=3.40$  d.f. = 1  $p<0.1$ ) with regard to low titres also almost significant ( $X^2=3.08$  d.f. = 1  $p<0.1$ ) and with regard to high titres not significant ( $X^2=0.40$  d.f. = 1  $p>0.1$ ). The difference between the blood donors and the sterile group with regard to incidence of positive sera was not

significant ( $X^2=1.59$   $df=1$   $p>0.1$ ) nor was the difference with regard to low titres ( $X^2=0.89$   $df=1$   $p>0.1$ ). The difference between the blood donors and the sterile group with regard to high titres was however highly significant ( $X^2=8.30$   $df=1$   $p<0.01$ ).

The earlier investigation showed that the sterile and fertile groups differed statistically significantly with regard both to the incidence of sperm agglutinin positive sera and to the incidence of high titres. The blood donor group reported on in this paper showed an incidence of sperm agglutinin positive sera that was just between those of the sterile and fertile groups. With regard to the incidence of high titres the blood donor group was more like the fertile group as the difference between the incidences of high titres of the blood donor group and the fertile group was small and statistically not significant while the difference with regard to high titres between the blood donor group and the sterile group was great and statistically highly significant.

Higher incidence of sperm agglutinin positive sera and sera with high titres was found in the sterile group in comparison to the incidence in the fertile group and the group not selected with regard to fertility. With regard to the incidence of low titres there were no statistically significant differences between the groups. The result of the investigation shows that there was an accumulation of men with sperm agglutinins especially of men with high titres, in the sterile group. This observation indicates that sperm antibodies in men, particularly at high levels, may interfere with fertility.

### SUMMARY

The sera of 500 male blood donors, 20-45 years old, were investigated for sperm agglutinins with Kibrick's gelatin agglutination test. No influence of the ABO blood factors in the differentiation of sperm agglutinin positive and negative groups was demonstrated. The incidence of positive sera was 4.8 per cent, 3.6 per cent with low titres ( $\leq 1:32$ ) and 1.2 per cent with high titres ( $\geq 1:64$ ). The incidence of high titres was significantly

Table IV *Distribution (Per Cent) of Sperm Agglutinin Negative Sera and Sera with Low ( $\leq 1/32$ ) and High ( $\geq 1/64$ ) Titres in the Sterile Fertile and Blood Donor Groups Absolute Numbers in ( )*

	Total	Negative Sera	Positive Sera		
			Low Titres	High Titres	Total
Sterile	100.0(400)	93.2	2.5	4.3	6.8
Fertile	100.0(500)	97.4	1.8	0.8	2.6
Blood donors	100.0(500)	95.2	3.6	1.2	4.8

The incidence of sperm agglutinin positivity was 6.8 per cent in the sterile and 2.6 per cent in the fertile group. The incidence in the blood donors was 4.8 per cent, or midway between the figures for the sterile and fertile groups.

In the earlier investigation it was found that the difference with regard to titre level between the sterile and the fertile groups was greatest if a limit was drawn between the titres  $1/32$  and  $1/64$ . Titres below this limit were called low and titres above high. The distribution of low and high titres in the sterile fertile and blood donor groups is given in Table IV. The sterile group had the greatest incidence of high titres. The fertile group showed the smallest incidence both of low titres and of high titres. The blood donor group showed the greatest incidence of low titres, the incidence of high titres in the blood donor group was somewhat greater than in the fertile group but considerably smaller than that of the sterile group.

Statistical analysis showed that the differences between the sterile and fertile groups were significant except in regard to low titres. A statistical analysis of the difference between the blood donors on one hand and the sterile and fertile groups on the other gave the following results. The difference between the blood donors and the fertile group with regard to incidence of positive sera was almost significant ( $\chi^2 = 3.40$  d.f. = 1  $p < 0.1$ ) with regard to low titres also almost significant ( $\chi^2 = 3.08$  d.f. = 1  $p < 0.1$ ) and with regard to high titres not significant ( $\chi^2 = 0.40$  d.f. = 1  $p > 0.1$ ). The difference between the blood donors and the sterile group with regard to incidence of positive sera was not

From the Institute of Medical Microbiology Department of Bacteriology (Professor O. Ouchterlony) University of Göteborg, and the Departments of Obstetrics and Gynecology II (Professor P. Bergman) Sahlgrenska sjukhuset Göteborg, Sweden

## CERVICAL MUCUS PENETRATION BY HUMAN SPERMATOZOA TREATED WITH ANTI-SPERMATOZOAL ANTIBODIES FROM RABBIT AND MAN

BY

BO FÄLLBRANT

### *Introduction*

Whether sperm antibodies have an anti-fertility effect has mostly been studied in female animals immunized with semen. In some experiments it has been shown that exposure of spermatozoa to sperm antibodies before insemination can prevent fertilization of ova. Kiddy *et al.* (1959) found that treatment of rabbit semen with high concentrations of anti-rabbit semen immune serum prevented fertilization and that the fertilization rate increased as lower antibody concentrations were used. Menge *et al.* (1962) reported that immune sera produced against bull semen and bull spermatozoa had an anti fertility effect when used to treat bull semen prior to insemination of heifers. Menge and Protzman (1967) showed that immune sera against rabbit testis, epididymal spermatozoa and semen prevented fertilization in rabbits, but immune sera against rabbit seminal plasma did not. The mechanism of the anti fertility effect was discussed but not definitively explained in these reports. In other experiments a decreased metabolic activity of antibody-treated spermatozoa was observed, e.g. by Matousek (1964) who found that the fructolysis of bull seminal spermatozoa was reduced by antibodies against seminal and epididymal bull spermatozoa and by Mizel *et al.* (1965) who found that antisera against bull semen reduced the fructose



lower ( $p < 0.01$ ) in the blood donor group than in a group of men in sterile marriages investigated earlier with the same technique and the same semen donor

### Acknowledgements

The Blood Bank (Chief Dr L. Ryttinger) Sahlgrenska sjukhuset, supplied the blood specimens and performed the blood group testing

### REFERENCES

- Bandhauer K. Immunreaktionen bei Fertilitätsstörungen des Mannes Urol. int (Basel) 21 247 1966
- Fjällbrant B. Immunoagglutination of sperm in cases of sterility Acta obstet. gynec. scand. 44 474 1965
- Fjällbrant B. Sperm agglutinins in sterile and fertile men. Acta obstet. gynec. scand. 47 89 1968
- Kibrick S. Belding, D. L. and Merrill B. Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. Fertil. and Steril 3 430 1952
- Phadise A. M. and Padukone A. Presence and significance of autoantibodies against spermatozoa in the blood of men with obstructed vas deferens. J. Reprod. Fertil 7 163 1964
- Rümke P. The presence of sperm antibodies in the serum of two patients with oligozoospermia. Vox Sang (Basel) 4 135 1954
- Rümke P. and Hellings G. Autoantibodies against spermatozoa in sterile men. Amer J clin. Path. 32 357 1959
- Schwimmer W. B. Utsey K. A. and Behrman S. J. An evaluation of immunologic factors of infertility Fertil. and Steril. 18 167 1967
- Wilson L. Sperm agglutinins in human semen and blood. Proc. Soc. exp. Biol (N.Y.) 85 652, 1954
- Wilson L. Sperm agglutination due to autoantibodies. A new cause of sterility Fertil. and Steril 7 262, 1956

Received on Feb 8 1968

Table I. Sperm Density, Percentage of Motile Spermatozoa and Sperm Motility of the Elucidates

Donor	Sperm Density (millions/ml)	Motile Spermatozoa (%)	Sperm Motility
1	120	60	very rapid
1	135	55	very rapid
1	110	55	very rapid
2	114	65	rapid
3	24	35	slow
4	75	85	very rapid
5	92	65	very rapid
6	102	70	apid
7	36	50	rapid

Seminal spermatozoa were washed 10 times with phosphate-buffered isotonic saline 0.1 M, pH 7.4 called buffered saline below. Then the spermatozoa were diluted to a density of 130 millions/ml with buffered saline and subsequently freeze-pressed 5 times in the x-press described by Edebo (1960). This preparation was used for the immunization of 2 rabbits.

Spermatocele contents was drawn by puncture from one patient and centrifuged at  $1500 \times g$  for 15 min. most of the supernatant was decanted and the rest transferred with the sediment to a smaller tube and recentrifuged at  $650 \times g$  for 15 min. The sedimented spermatozoa were washed 3 times with buffered saline diluted to 200 millions/ml and x-pressed 5 times. This preparation was used for immunization of another 2 rabbits.

The described seminal preparations were kept in small portions at  $-30^{\circ}C$  until used as antigen.

For preparation of rabbit antisera injections were given subcutaneously and intravenously. The subcutaneous injections consisted of 0.5 ml seminal plasma, 0.5 ml of the suspension of seminal spermatozoa and 1.0 ml of the suspension of spermatocele spermatozoa respectively emulsified in an equal volume of Freund's complete adjuvant (paraffin oil + Arlatel® + mycobacteria). The schedule implied weekly subcutaneous injections for 6 weeks, the last week supplemented with 3 intravenous injections.

utilization the oxygen uptake and the lactic acid production of bull spermatozoa

No studies on the fertilization capacity of antibody treated spermatozoa have been performed in man and will probably not be done except as chance ordains. Wilson (1954-1956) in studies of three men with sperm antibodies observed that the spermatozoa quickly lost their motility in cervical mucus at postcoital and *in vitro* tests. In a more extensive study on men with sperm antibodies Fjällbrant (1968 b) found an interrelation between high levels of sperm antibodies in blood reduced penetration of cervical mucus by their spermatozoa and sterility. The investigation did not show however that the reduction of the penetration ability and the sterility were actually caused by the sperm antibodies.

The aim of the present investigation was to study the mucus penetration ability of human spermatozoa treated with rabbit antisera against spermatozoa and seminal plasma and with sera from men with naturally occurring sperm antibodies.

### *Material*

#### *Spermatozoa*

Semen samples from 7 donors without sperm antibodies were used. All donors were married and had become fathers within the previous 5 years. Six of them had 2 or more children. Sperm density, percentage of motile spermatozoa and sperm motility of the semen samples are given in Table I. Three ejaculates from donor 1 were used for experimental series I-III and one ejaculate from each of donors 2-7 for series IV.

#### *Rabbit antisera*

A pool of normal ejaculates from 40 donors which had been kept at  $-30^{\circ}\text{C}$  was centrifuged at  $330 \times g$  for 15 min and the spermatozoa harvested. The supernate was recentrifuged at  $2000 \times g$  for 30 min.

Seminal plasma decanted after the recentrifugation was used for immunization of 4 rabbits.

Table II. Sperm Agglutinin Titre and Sperm Immobilizing Activity of the Sera Used for Treatment of Spermatozoa

Serum	Agglutinin Titre	Immobilizing Activity
Rabbit control serum	0	8 h. 15 min.
Anti-venereal plasma serum	1 65,536	12 min.
Anti-venereal spermatozoa serum	1 8 192	15 min.
Anti-spermatozoa spermatozoa serum	1 1,024	55 min.
Human male control serum	0	60 h.
Patient 1 (sterile)	1 128	3 h. 15 min.
Patient 2 (sterile)	1 256	4 h.
Patient 3 (sterile)	1 2,048	4 h. 30 min.
Patient 4 (sterile)	1 8	30 h.
Patient 5 (fertile)	1 8	38 h.
Patient 6 (fertile)	1 16	42 h.

measure of immobilizing activity of a serum was the time required to reduce the percentage of motile spermatozoa from about 70 to 10 in the presence of complement. The same semen donor as for the agglutinin titre determinations was used.

#### Determination of mucus penetration by spermatozoa

The cervical mucus penetration of spermatozoa was determined with the method of Kremer (1965) slightly modified. Cervical mucus samples were taken from women at the expected time of ovulation. The penetrability was tested with semen from the donor used for the sperm antibody tests. Selected for use were 10 readily penetrated voluminous samples of mucus one from each of 10 women. The mucus samples were transparent and had a high spinbarkeit and such a low viscosity that the mucus could be easily drawn up into capillary tubes. A column of cervical mucus, about 40 mm long, was drawn into a capillary tube the inner diameter of which was 0.7 mm. The ends of the tube were sealed with modeling clay. The sealed tube was stored at +4 °C until used some days later. Then one of the seals was removed and the other partly pushed into the capillary tube so that a mucus

tions of the preparation (0.25, 0.25 and 0.50 ml, respectively) given every other day. One week after the last injection the animals were bled. Two rabbits were injected only with adjuvant. Pre-immunization bleedings were taken. The sera were investigated for sperm agglutinins, sperm immobilizing antibodies and precipitating antibodies. Sera from the pre-immunization bleedings and sera from the rabbits injected with adjuvant only had no agglutinating or precipitating antibodies and showed a low sperm immobilizing activity. The antiserum against each preparation which showed the highest agglutinin titre, the greatest immobilizing activity and the largest number of lines in double diffusion-in-gel analysis was selected for this study. In the mucus penetration studies a pre-immunization bleeding was used as a control (rabbit control serum). The sperm agglutinin titres and the sperm immobilizing activities of the used sera are given in Table II.

#### *Patient sera*

Blood was taken from 4 male patients in the sterility clientele of the clinic who were known to have sperm antibodies in the blood and from 2 fertile men with sperm antibodies. Blood from one fertile man without sperm antibodies was used as a control (human male control serum). After centrifugation of the blood the decanted sera were kept at  $-30^{\circ}\text{C}$  until used. The sperm agglutinin titre and the sperm immobilizing activity of the sera were determined. These values are given in Table II.

### *Methods*

#### *Determination of sperm agglutinin titre*

The sperm agglutinin titre of both the rabbit and human sera was determined with the macroscopic direct sperm agglutination method of Kibrick *et al.* (1952) slightly modified as described in an earlier paper (Fjällbrant 1968a). Semen from one donor was used for all the determinations.

#### *Determination of sperm immobilizing activity*

The sperm immobilizing activity of the sera was determined as described in an earlier paper (Fjällbrant 1968b). The employed

Table III *Cervical Mucus Penetration and Motility of Mucus Spermatozoa from Donor 1 Exposed to Various Antiser*

Test Mixture	Penetration	Sperm Motility in Mucosa	
Series I a. Rabbit antisera at sperm-aggglutinating concentration			
	Mucus 1	Mucus 2	
Semen	≥ 30 mm	≥ 30 mm	very rapid
Semen rabbit control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen anti-serumal plasma serum	≤ 5 mm	≤ 5 mm	in loco
Semen anti-serumal spermatozoa serum	≤ 5 mm	≤ 5 mm	in loco
Semen anti-spermatozoale spermatozoa serum	10 mm	14 mm	in loco
Series I b. Rabbit antisera at non-aggglutinating concentration			
	Mucus 3	Mucus 4	
Semen	≥ 30 mm	≥ 30 mm	very rapid
Semen rabbit control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen anti-serumal plasma serum	≥ 30 mm	≥ 30 mm	in loco
Semen anti-serumal spermatozoa serum	≥ 30 mm	≥ 30 mm	in loco
Semen anti-spermatozoale spermatozoa serum	≥ 30 mm	≥ 30 mm	in loco
Series II Human sera with sperm antibodies at high titre			
	Mucus 5	Mucus 6	
Semen	≥ 30 mm	≥ 30 mm	very rapid
Semen human male control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen patient serum 1	≤ 5 mm	≤ 5 mm	in loco
Semen patient serum 2	≤ 5 mm	≤ 5 mm	in loco
Semen patient serum 3	≤ 5 mm	≤ 5 mm	in loco
Series III Human sera with sperm antibodies at low titre			
	Mucus 7	Mucus 8	
Semen	≥ 30 mm	≥ 30 mm	very rapid
Semen human male control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen patient serum 4	20 mm	27 mm	in loco
Semen patient serum 5	20 mm	23 mm	slow
Semen patient serum 6	≥ 30 mm	≥ 30 mm	rapid

drop the size of a pinhead was formed at the other end. This end was immersed into a chamber containing the semen to be tested. After 3 hours in a moist atmosphere at  $+37^{\circ}\text{C}$  the extent of penetration was read microscopically. A penetration  $\geq 30$  mm was classified as normal a penetration 6–29 mm as moderately reduced and a penetration  $\leq 5$  mm as highly reduced.

The motility of the spermatozoa which had penetrated into the mucus was registered as very rapid rapid or slow movement forward or as motility only *in loco* without forward movement.

### Experimental

Portions of semen were distributed to small precipitation tubes and mixed with test serum. Besides the antisera one rabbit or human control serum was used in each series as well as one untreated semen portion. The tubes were incubated at  $+37^{\circ}\text{C}$  for 1 hour. After incubation a drop of the contents was studied by microscopy to estimate motility and agglutination of the spermatozoa and the rest was transferred to the semen chamber for determination of cervical mucus penetration of the spermatozoa.

The results are summarized in Tables III and IV. In all of the series the untreated spermatozoa and the spermatozoa mixed with the control sera showed normal penetration and the motility in mucus was rapid or very rapid with the exception of one semen sample (donor 3). In series 1–3 the penetration of the spermatozoa of each semen portion treated or untreated was tested with mucus from two different females for each portion the results were similar with the two mucus samples. In series 4 each semen portion was tested with one mucus sample.

*Series I a Spermatozoa from one donor treated with various rabbit antisera at sperm-agglutinating concentration (Table IV)*

To 4 aliquots of 0.4 ml semen from donor 1 was added 0.03 ml anti seminal plasma serum 0.1 ml anti seminal spermatozoa serum 0.1 ml anti-spermatocyte spermatozoa serum and 0.1 ml rabbit control serum respectively. These volumes were chosen because the antisera added to the semen in these proportions had

*Series II. Spermatozoa from one donor treated with human sera with sperm antibodies at high titre (Table III)*

In this series 0.2 ml serum from each of 3 patients with high sperm antibody levels was added to aliquots of 0.2 ml semen from donor 1. After incubation there was a moderate agglutination and a clearly reduced sperm motility.

The results were uniform as all the exposed spermatozoa had a highly reduced penetration and a motility in mucus only *in loco*.

*Series III. Spermatozoa from one donor treated with human sera with sperm antibodies at low titre (Table III)*

In this series 0.2 ml of serum from each of 3 men with low sperm antibody levels was added to 0.2 ml aliquots of semen from donor 1. After incubation there was no agglutination and a very rapid sperm motility.

The spermatozoa exposed to the serum which had the lowest immobilizing activity (no. 6) had normal penetration, while the spermatozoa of the other serum mixtures had a moderately reduced penetration. The serum from the sterile man (no. 4) had the highest immobilizing activity and the spermatozoa treated with this serum had a motility in mucus only *in loco* while the spermatozoa treated with serum from patient 5 had a slow and the spermatozoa treated with serum from patient 6 had rapid movement forward in mucus.

*Series IV. Spermatozoa from various donors treated with rabbit and human antiserum (Table IV)*

To 0.4 ml semen from donors 2-7 was added 0.03 ml anti-seminal plasma serum, and to 0.2 ml semen from the same donors was added 0.2 ml serum from one of the patients with a high sperm antibody level (no. 1). After incubation all the mixtures showed tail-to-tail agglutination. The motility was decreased, but to a lesser degree in those samples where the initial motility was very rapid and the percentage of motile spermatozoa high.

With the exception of the semen aliquot from the donor with the highest percentage of motile spermatozoa (no. 4) which was



Table IV *Series IV Cervical Mucus Penetration and Motility in Mucus of Spermatozoa from Different Donors Exposed to Rabbit Antiserum Against Seminal Plasma or Serum from a Man with Sperm Antibodies*

Donor	Semen		Semen + Anti-seminal Plasma Serum		Semen + Serum from Patient 1	
	Penetration	Motility in Mucus	Penetration	Motility in Mucus	Penetration	Motility in Mucus
2	≥ 30 mm	rapid	≤ 5 mm	in loco	≤ 5 mm	in loco
3	≥ 30 mm	slow	≤ 5 mm	none	≤ 5 mm	none
4	≥ 30 mm	very rapid	≥ 30 mm	in loco	23 mm	in loco
5	≥ 30 mm	very rapid	12 mm	in loco	8 mm	in loco
6	≥ 30 mm	rapid	≤ 5 mm	in loco	≤ 5 mm	in loco
7	≥ 30 mm	rapid	≤ 5 mm	in loco	≤ 5 mm	in loco

Comment: Donors 2-5 were tested with mucus no. 9 and donors 6 and 7 with mucus no. 10.

proved to cause a moderate agglutination of the tail to-tail type but to leave most of the spermatozoa free with rather rapid motility after incubation for 1 hour at +37 °C.

The spermatozoa exposed to anti-seminal plasma serum and anti-seminal spermatozoa serum showed a highly reduced penetration and the spermatozoa exposed to anti-spermatocyte spermatozoa serum had a moderately reduced penetration. The spermatozoa in all the antiserum mixtures had a motility in mucus only *in loco* without forward movement.

#### *Series I b Spermatozoa from one donor treated with various rabbit antisera at non-agglutinating concentration (Table III)*

Only one-tenth of the volume of each of the rabbit antisera used in series I a was added to the aforementioned volume of semen from donor 1. After incubation there was no sperm agglutination and very rapid sperm motility.

The mucus penetration was normal. The motility of the spermatozoa in mucus was however reduced to motility only *in loco*.

*Series II. Spermatozoa from one donor treated with human sera with sperm antibodies at high titre (Table III)*

In this series 0.2 ml serum from each of 3 patients with high sperm antibody levels was added to aliquots of 0.2 ml semen from donor 1. After incubation there was a moderate agglutination and a clearly reduced sperm motility.

The results were uniform as all the exposed spermatozoa had a highly reduced penetration and a motility in mucus only *in loco*.

*Series III. Spermatozoa from one donor treated with human sera with sperm antibodies at low titre (Table III)*

In this series 0.2 ml of serum from each of 3 men with low sperm antibody levels was added to 0.2 ml aliquots of semen from donor 1. After incubation there was no agglutination and a very rapid sperm motility.

The spermatozoa exposed to the serum which had the lowest immobilizing activity (no. 6) had normal penetration, while the spermatozoa of the other serum mixtures had a moderately reduced penetration. The serum from the sterile man (no. 4) had the highest immobilizing activity and the spermatozoa treated with this serum had a motility in mucus only *in loco* while the spermatozoa treated with serum from patient 5 had a slow and the spermatozoa treated with serum from patient 6 had rapid movement forward in mucus.

*Series IV. Spermatozoa from various donors treated with rabbit and human antiserum (Table IV)*

To 0.4 ml semen from donors 2-7 was added 0.03 ml anti-seminal plasma serum, and to 0.2 ml semen from the same donors was added 0.2 ml serum from one of the patients with a high sperm antibody level (no. 1). After incubation all the mixtures showed tail-to-tail agglutination. The motility was decreased but to a lesser degree in those samples where the initial motility was very rapid and the percentage of motile spermatozoa high.

With the exception of the semen aliquot from the donor with the highest percentage of motile spermatozoa (no. 4) which was

treated with antiserinal plasma serum the penetration was reduced in all cases. In four cases (nos 2 3 6 and 7) it was highly reduced when the semen samples were treated with either the rabbit antiserum or the patient serum. With both antisera the motility in mucus was reduced to a motility *in loco* in five cases (nos 2 and 4-7). The spermatozoa from the donor with the lowest percentage of motile spermatozoa and the least motility (no 3) showed no motility in mucus.

### *Discussion and Conclusions*

The effect on penetration of selected cervical mucus by treatment of spermatozoa in normal ejaculates from one donor with different volumes of rabbit antiserum against seminal plasma, seminal spermatozoa and spermatocele spermatozoa and with serum from men with different levels of sperm antibodies was studied. To investigate whether and to what extent spermatozoa from other men were also affected by sperm antibodies semen samples from six other fertile men were treated with rabbit anti seminal plasma serum and with serum from a man with sperm antibodies.

The suitability of the methods for the determination of sperm agglutinin titre and sperm immobilizing activity of sera has been discussed in earlier papers (Fjällbrant 1968 a and b) as has the applicability of the method for the determination of mucus penetration of spermatozoa (Fjällbrant 1968 b). The method used for immunization of the rabbits provided antisera with very high sperm agglutinating and immobilizing activities. Of the rabbit antisera the anti seminal plasma antiserum had the greatest agglutinating and immobilizing activity. The anti seminal spermatozoa serum had a lesser activity and least was the activity of the serum against spermatocele spermatozoa. There was a similar trend of differences in precipitin content as noted in the double diffusion-in gel patterns which will be reported on in a separate paper.

Because both the untreated spermatozoa and the spermatozoa treated with control sera showed normal penetration and unimpaired motility in mucus it can be assumed that the reduction of penetration and motility observed in spermatozoa treated with antisera was caused by antibodies in the immune sera.

With regard to the discussion on the antigenicity of seminal plasma versus spermatozoa it is notable that antisera against seminal plasma, seminal spermatozoa as well as spermatocele spermatozoa had immobilizing and agglutinating activity and caused reduction of penetration of the spermatozoa treated. Although the anti-seminal plasma serum contained some antibodies against spermatozoa (demonstrated with diffusion-in-gel technique) its very high agglutinating, immobilizing and penetration-reducing activity must presumably be referred also to its high content of antibodies against secretions from the male adnexal glands. As these penetration-reducing antibodies might be supposed to prevent spermatozoa from fertilizing ova the finding of penetration-reducing antibodies against human seminal plasma is in certain respects opposite to the finding of Menge and Protzman (1967) that rabbit testicular material and not seminal plasma was the source of antigens that induced antibodies capable of preventing spermatozoa from fertilizing ova in does. From the present investigation it seems evident that human spermatozoa *per se* can elicit production of sperm agglutinating, immobilizing and penetration-reducing antibodies, because the spermatocele spermatozoa used had not been in contact with seminal plasma.

Volumes of rabbit antisera were added to the semen aliquots to cause only a moderate agglutination and a moderate reduction of sperm motility. Although most of the spermatozoa had a rather rapid motility after incubation the mucus penetration of the treated spermatozoa was highly reduced. When the same volumes of semen were treated with one tenth of these immune serum volumes the spermatozoa seemed to be unaffected after incubation, but still there was an obvious decrease of motility in the cervical mucus although the penetration was normal. The sera from male patients which contained sperm agglutinating and immobilizing antibodies (but no detectable precipitins) had largely the same effect on spermatozoa as the rabbit antisera. Sera with high antibody concentrations considerably reduced the penetration and the motility of the spermatozoa in mucus. Sera with low antibody concentrations reduced the penetration and the motility to a lesser degree which was proportional to the immobilizing activity of the sera. Thus it seems clear that the reduction of the

penetration of antibody treated spermatozoa depends on the concentration of the antibodies

When semen aliquots from six different fertile donors were treated with equal amounts of rabbit antiserum or patient serum, both containing sperm antibodies the spermatozoa from all of the donors were affected but not to the same extent. The motility in mucus was strongly decreased in all cases, and the penetration was highly reduced in four cases. The spermatozoa from the donor who had the highest percentage of motile spermatozoa and very rapid sperm motility had however normal penetration after treatment with the rabbit antiserum and moderately reduced penetration after treatment with patient serum. The penetration of spermatozoa from the other donor who had very rapid sperm motility was moderately reduced by both types of antisera. Thus the reduction of mucus penetration by antibody treated spermatozoa appears to depend not only on the antibody concentration but also on intrinsic properties of the spermatozoa.

In an earlier investigation (Fjällbrant 1968 c) it was found that spermatozoa from men with sperm antibodies showed degrees of motility reduction and agglutination which could be correlated to the sperm antibody level in the blood. The spermatozoa had a reduced mucus penetration which could also be correlated to the sperm antibody level in the blood (Fjällbrant 1968 b). From the present investigation it seems clear that normal spermatozoa treated with antibodies behave similarly to spermatozoa from men with sperm antibodies in the blood.

The results of the present investigation show that antibodies against human spermatozoa as well as antibodies against seminal plasma both those produced in rabbits and those naturally occurring in males cause a reduction of the mucus-penetrating ability of spermatozoa. The degree of impairment seems to depend on the antibody concentration as well as on the intrinsic properties of the spermatozoa. Reduction of the penetration ability of spermatozoa diminishes the possibility of spermatozoa reaching the ovum and fertilizing it. Although there are other conceivable ways for sperm antibodies to render spermatozoa incapable of fertilization the reduction of penetration ability of spermatozoa seems to be a likely mechanism for sperm antibodies to cause sterility in men.

## SUMMARY

Semen aliquots from one donor were treated with different volumes of rabbit antisera against seminal plasma seminal spermatozoa and spermatocele spermatozoa and with sera from men with different concentrations of sperm antibodies in the blood. The penetration of the antibody-treated spermatozoa in selected cervical mucus was investigated. Low concentrations of sperm antibodies reduced the sperm motility in mucus and high concentrations reduced both the sperm motility in mucus and the extent of sperm penetration. When semen from six fertile donors was treated with rabbit antiserum against seminal plasma and with serum from a man with high sperm antibody concentration the spermatozoa from all the donors were affected, but the penetration of spermatozoa from ejaculates with high percentages of motile spermatozoa and very rapid sperm motility was less reduced. The results of the investigation show that antibodies against human spermatozoa as well as antibodies against seminal plasma both those produced in rabbits and those naturally occurring in males, cause reduction of the cervical mucus-penetrating ability of spermatozoa. The degree of impairment depends on the antibody concentration and the intrinsic properties of the spermatozoa. Reduction of mucus penetration ability of spermatozoa seems to be a likely mechanism for sperm antibodies to cause sterility in men.

*Acknowledgements*

Miss Karin Larén and Miss Yvonne Steen performed as technical assistants. The English text was revised by Translator Charles Wadsworth. The investigation was supported by a grant from the Faculty of Medicine University of Göteborg.

## REFERENCES

1. Ishio I. A new press for the disruption of micro-organisms and other cells. *J. Biochem. Microbiol. Technol. Eng.* 2: 453, 1960.
2. Lillqvist B. Sperm agglutination in sterile and fertile men. *Acta obstet. gynec. scand.* 47: 39, 1968.

- Fjällbrant B., Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility in men *Acta obstet. gynec. scand.* 47 102, 1968
- Clinical and seminal findings in men with sperm antibodies. *Acta obstet. gynec. scand.* 47 451 19 8
- Isbrick S. Belding, D. L. and Merrill B. Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. *Fertil. and Steril.* 3 430 1952
- Kiddy C. A. Stone W. H. and Casida L. E. Immunologic studies on fertility and sterility II. Effects of treatment of semen with antibodies on fertility in rabbits. *J. Immunol.* 87 125 1959
- Kremer J. A simple sperm penetration test. *Int. J. Fertil.* 10 209 1965
- Matousek J. Antigenic characteristics of spermatozoa from bulls, rams and boars. III. Absorption analysis precipitins and fructolysis in relation to the antigenicity of bull spermatozoa. *J. Reprod. Fertil.* 8 13 1964
- Menge A. C. Stone W. H. Tyler W. J. and Casida L. E. Immunological studies on fertility and sterility IV. Fertility of cattle and rabbits inseminated with semen treated with antibodies produced against semen, spermatozoa and erythrocytes. *J. Reprod. Fertil.* 3 331 1962
- Menge A. C. and Protzman W. P. Origin of the antigens in rabbit semen which induce antifertility antibodies. *J. Reprod. Fertil.* 13 31 1967
- Mittal A. K. Salisbury G. W. Graves C. N. and Rasmussen B. A., Antigens of bovine semen and the influence of specific rabbit anti-bull-semen serum on metabolic activity of bull spermatozoa. *J. Reprod. Fertil.* 10 29 1965
- Wilson L. Sperm agglutinins in human semen and blood. *Proc. Soc. exp. Biol. (N. Y.)* 85 652, 1954
- Sperm agglutination due to autoantibodies. A new cause of sterility *Fertil. and Steril.* 7 262 1956

Received on Feb. 14 1968

## TREATMENT OF TRICHOMONAS VAGINITIS WITH NIFURATEL

BY

H. GJØNNÆSS AND J. CHR. AURÉ

*Trichomonas vaginalis* infections are a daily problem for most gynaecologists. In unselected material from Reims (Chappaz and Bertrand 1965) trichomonas infections were found in 10 per cent of 1717 pregnant patients a similar incidence was found in Manchester (Vart 1965).

Trichomonas infections occur most often among women of childbearing age but also can appear after the menopause in young girls or even in the new-born (Dagenals-Pétrusse et al. 1964).

There is little doubt that trichomonas vaginalis can be transmitted at coitus. Chappaz and Bertrand (1965) refer to various papers in which trichomonas vaginalis infections have been found in the male partner in 0-100 per cent of cases.

The importance of systematic therapy as opposed to simply local treatment in the vagina is now generally accepted. Chappaz and Bert and (1965) noted that in 292 women with vaginal infections, trichomonas vaginalis was also present in the urethra in 190 (65 per cent). In 505 Norwegian patients with trichomonas agnatus the organism was discovered in the urethra in 80.8 per cent and in the cervix in 79.4 per cent (Ødegaard, 1962 a).

Experience over many years has confirmed the failure of treatment of vaginal trichomonas infections using local therapy alone. The appearance of metronidazole therefore represented an advance in one could now use a preparation for systemic treatment. The effectiveness of metronidazole seems to be proven in

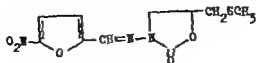


several large series the frequency of successfully treated cases has been shown to be between 80 per cent and 99 per cent (Warr and Jennison 1962 Sharman 1963 Peterson *et al* 1966)

The problem of *trichomonas vaginalis* developing resistance to therapy has been investigated. Ødegaard (1962 b) tried in vain to produce an increased *in vitro* resistance by culturing *trichomonas vaginalis* in media with low concentrations of metronidazole De Camerl (1966) however succeeded with a somewhat similar technique Jennison *et al* (1961) found no increased resistance to metronidazole in *trichomonas vaginalis* from patients who had undergone one course of therapy Arnold (1966) reported a success rate of 92.6 per cent in 140 patients treated in 1960-61 but in 1965-66 using a presumably similar method of treatment in a comparable group of 72 patients he found a cure rate of only 68 per cent Svendsen (1962) in a series of 76 patients reported a cure rate of 97 per cent In a newly completed study from the same area the success rate in 120 patients was 73 per cent (Aure and Gjonnaess) Whereas some authors maintain that the reduced effectiveness must be due to the failure of the active substance in reaching the organisms the results from Switzerland and Norway imply that one should be prepared for possible development of resistance against metronidazole

A new compound has been synthesized by Polichimica Sap Milano which may offer alternative therapy thus avoiding the problem of resistance and thereby improve the cure rate. The substance is a nitrofurant derivative with the generic name nifuratel (Fig 1) Nifuratel is a yellow crystalline substance difficult to dissolve in water or the usual organic solvents insoluble in mineral acids and alkali but soluble in dimethylformamide Extensive investigations have shown that nifuratel is practically non toxic both after short and long term administration (Scuri 1966) There were no effects shown on various organic functions on haemopoiesis spermatogenesis or ovulation. There was no teratogenic effect demonstrable in mice rats or rabbits and no tendency to accumulate *In vitro* studies carried out at the University of Pisa (Imperato 1963) showed a marked trichomon-

In the Scandinavian countries the preparation will be marketed under the name Polmirus<sup>®</sup>



5- [(methylthio)ethyl]-3-  
 [(5-nitrofurfurylidene)amino]-  
 2-oxaxolidinone

Fig. 1 Nifuratel.

cidal effect (total inhibition of growth with a concentration of 0.25 MCG/cc) *In vivo* trials (intraperitoneal inoculation with trichomonas in mice) showed a good trichomonocidal effect both when nifuratel was administered orally and directly into the peritoneal cavity. It was also shown that nifuratel was effective against various Gram positive and Gram negative bacteria, and also in higher concentrations against various fungi, including 3 strains of *Candida*.

Several clinical studies have been published and some of these are shown in Table I. Regarding the dosage of nifuratel, the results of Arnold (1966) and Vartiainen and Widholm (1966) imply that it is important to achieve a high vaginal concentration. Arnold achieved the best results with either a combined treatment (1 tablet of 0.2 g twice daily + 1 vaginal tablet of 0.45 g twice daily both for 10 days) or with vaginal treatment (1 vaginal tablet twice daily for 20 days) alone. Both these schemes gave a success rate of 85 per cent. Sarnella (1966) administered an oral dose of 0.2 g three times daily for 7 days + 1 vaginal tablet daily for 15 days and achieved a success rate of 90 per cent. The conclusion seems to be that combined oral and vaginal treatment yields the best result. Almost as good results can be achieved with ample vaginal treatment, but these patients have a higher recurrence rate.

It was decided to evaluate the effectiveness of nifuratel in a controlled population and observe differences in response to dosage and treatment failures.

Table I. *Nifuratel—Clinical Reports*

Author	Department	Year	Patients	Treatment	Cure Rate
Arnold M.	Universitätsfrauen- klinik Bern	1966	485	oral vaginal comb	64 " 83-93 " 75-91 "
Leon R.	Obstetric and Gynecologic Clinic University of Florence	1964	30	comb	93 %
Sagone I.	Obstetric and Gynecologic Clinic University of Milan	1965	61 20	oral comb	82 % 70 "
Sarnella, A.	Obstetric and Gynecologic Clinic University of Siena	1966	400	comb.	95 %
Vartiainen E. and Widholm O.	Central Hospital University of Helsinki	1966	15 20	oral vaginal	47 " 80 "

All results are primary control examinations 1-3 months later where done. In all reports revealed less favourable results.

In none of the reports was there mention of side effects except for 1 case with vasomotor reaction after consumption of alcohol.

### *Material and Methods*

The series consists of all patients with *trichomonas vaginalis* infections seen in the gynaecological out-patient department at Lillehammer from March 1966 to January 1967. In most cases the diagnosis was verified by direct microscopy on fresh preparations stained with 0.25 % Brilliant-Cresyl blue in isotonic saline. This solution stains leucocytes and epithelial cells but not *trichomonas vaginalis*. They still exhibit approximately the same degree of motility as in isotonic saline solution.

An attempt was made to carry out the treatment continuously without interruptions due to menstruation. In two patients this was not successful as menstruation occurred earlier than expected and their course of treatment was temporarily discontinued during menstruation. The other patients were examined at least 10 days after the end of the course of therapy. All patients received instructions to return if they suspected a recurrence. In no

Table II. Age Distribution

Age Years	Number of Patients
16-19	11
20-29	36
30-39	22
40-49	19
50-59	7
Total	95

patient was the follow-up period less than 2 months. Those patients in whom trichomonas could be demonstrated within the first 4 months, even if the primary treatment were successful were termed not cured.

The patients were divided into two groups, both receiving nifuratel. Therapy was given in two courses, I and II. The first group (60 patients) received 1 oral tablet (0.2 g) three times daily for 7 days, and the second group (30 patients) 1 oral tablet three times daily for 10 days. Both groups received 1 vaginal tablet (0.25 g) every evening for 10 days. The division into groups was carried out randomly irrespective of clinical factors such as coexistent cervicitis. (The term cervicitis is here meant to describe a condition with muco-purulent discharge possible erosion of the vaginal cervix and subjective discomfort in the form of pressure—tension and pain in the abdomen.)

The second course of therapy consisted of 1 oral tablet three times daily for 10 days together with 1 vaginal tablet twice daily for 10 days. In all cases it immediately followed the examination which revealed persistent live trichomonas vaginalis. An attempt was made to give therapy to all sexual partners but for various reasons only 67 were treated, as shown in Table V. The partner received the same number of oral tablets as the patient.

$\chi^2$ -test was used to determine if there were any statistical difference in the results between the different groups.

Table I *Nifuratel—Clinical Reports*

Author	Department	Year	Patients	Treatment	Cure %
Arnold M.	Universitätsfrauen- klinik Bern	1966	465	oral vaginal comb.	64 " 83-92 75-91
Leon R.	Obstetric and Gynecologic Clinic University of Florence	1964	30	comb.	93 "
Sagone I.	Obstetric and Gynecologic Clinic University of Milan	1965	61 20	oral comb.	87 " 70 "
Sarnella A.	Obstetric and Gynecologic Clinic University of Siena	1966	400	comb.	95 "
Vartiainen E. and Widholm O.	Central Hospital University of Helsinki	1966	15 20	oral vaginal	47 " 80 "

All results are primary control examinations 1-3 months later where done in all reports revealed less favourable results.

In none of the reports was there mention of side effects, except for 1 case with vasomotor reaction after consumption of alcohol.

### *Material and Methods*

The series consists of all patients with trichomonas vaginalis infections seen in the gynaecological out-patient department at Lillehammer from March 1966 to January 1967. In most cases the diagnosis was verified by direct microscopy on fresh preparations stained with 0.25 % Brilliant-Cresyl blue in isotonic saline. This solution stains leucocytes and epithelial cells but not trichomonas vaginalis. They still exhibit approximately the same degree of motility as in isotonic saline solution.

An attempt was made to carry out the treatment continuously without interruptions due to menstruation. In two patients this was not successful as menstruation occurred earlier than expected and their course of treatment was temporarily discontinued during menstruation. The other patients were examined at least 10 days after the end of the course of therapy. All patients received instructions to return if they suspected a recurrence. In no

Table II. Age Distribution

Age Years	Number of Patients
16-19	11
20-29	36
30-39	22
40-49	19
50-59	7
Total	95

patient was the follow-up period less than 2 months. Those patients in whom trichomonas could be demonstrated within the first 4 months even if the primary treatment were successful were termed not cured.

The patients were divided into two groups, both receiving nifuratel. Therapy was given in two courses I and II. The first group (60 patients) received 1 oral tablet (0.2 g) three times daily for 7 days, and the second group (30 patients) 1 oral tablet three times daily for 10 days. Both groups received 1 vaginal tablet (0.25 g) every evening for 10 days. The division into groups was carried out randomly irrespective of clinical factors such as coexistent cervicitis. (The term cervicitis is here meant to describe a condition with muco-purulent discharge possible erosion of the vaginal cervix and subjective discomfort in the form of pressure—tension and pain in the abdomen.)

The second course of therapy consisted of 1 oral tablet three times daily for 10 days together with 1 vaginal tablet twice daily for 10 days. In all cases it immediately followed the examination which revealed persistent live trichomonas vaginalis. An attempt was made to give therapy to all sexual partners, but for various reasons only 67 were treated as shown in Table V. The partner received the same number of oral tablets as the patient.

$\chi^2$ -test was used to determine if there were any statistical difference in the results between the different groups.

### Results

The results of the treatment can be seen in Table III. (In two instances the treatment was discontinued and three patients did not return for examination after the first course.) There was no difference in the results between the two groups. The duration of symptoms did not influence the results of treatment, neither did parity nor various hormonal states such as diabetes pregnancy, menstrual disorders or menopause.

Of 60 patients, who had vaginitis alone 49 were cured. Of 30 patients, who also had cervicitis 20 were cured (Table IV). This difference is not statistically significant.

In those cases where both sexual partners were treated, the success rate after one course was 73 per cent (49/67) (Table V). If the partner was not treated the success rate was 43 per cent (10/23). This difference is statistically significant ( $p < 0.05$ ). After two courses the success rate was 82 per cent (55/67) when the partner was treated, and 61 per cent (14/23) if he was not treated.

The effect of nifuratel in relation to other treatment can be seen in Table VI. The earlier treatment in most cases had been begun by other doctors and the unsatisfactory results of this therapy had motivated referral of the patient to hospital. Approximately half of the patients who were treated primarily with nifuratel or with metronidazole without effect responded when the other preparation was used. In the group of patients not cured with other treatment (= local treatment of different forms) the effect of nifuratel was approximately the same as overall. Of the 4 patients who failed to respond to nifuratel 3 had some benefit from metronidazole. Among the patients reported as uncured after 2 courses of nifuratel and 1 course of metronidazole there was one who was cured after a total of 3 courses of nifuratel and one after a total of 4 courses alternating with other forms of local treatment. Two patients received metronidazole orally and nifuratel vaginal tablets twice daily for 10 days and both were cured.

No serious side effects were recorded. Seven patients who previously had completely regular menstruation began menstruating 1 week too early. Urticaria occurred in 2 cases in one of

Table III. Results of Treatment with Nifuratel

Course I Oral Treatment (Days)	Course I			Course II			Cured After Two Courses	%
	N	Cured	Not Cured	N	Cured	Not Cured		
7	60	39	21	15	7	8	46	77
10	30	20	10	10	3	6	23	77

One patient was not controlled after course II.

Table IV. Results in Relation to Local Findings

	Course I			Course II			Cured After Two Courses	%
	N	Cured	Not Cured	N	Cured	Not Cured		
Vaginitis Cervicitis and Vaginitis	60	42	18	15	7	7	49	82
Vaginitis	30	17	13	10	3	7	20	67

One patient was not controlled after course II.

Table V. Results in Relation to Treatment of Sexual Partners

	Course I			Course II			Cured After Two Courses	%
	N	Cured	Not Cured	N	Cured	Not Cured		
Partners treated	67	49	18	14	6	7	53	82
Partners not treated	23	10	13	11	4	7	14	61

One patient was not controlled after course II.



### Results

The results of the treatment can be seen in Table III (In two instances the treatment was discontinued and three patients did not return for examination after the first course.) There was no difference in the results between the two groups. The duration of symptoms did not influence the results of treatment neither did parity nor various hormonal states such as diabetes pregnancy menstrual disorders or menopause.

Of 60 patients who had vaginitis alone, 49 were cured. Of 30 patients who also had cervicitis 20 were cured (Table IV). This difference is not statistically significant.

In those cases where both sexual partners were treated the success rate after one course was 73 per cent (49/67) (Table V). If the partner was not treated the success rate was 43 per cent (10/23). This difference is statistically significant ( $p < 0.05$ ). After two courses the success rate was 82 per cent (55/67) when the partner was treated and 61 per cent (14/23) if he was not treated.

The effect of nifuratel in relation to other treatment can be seen in Table VI. The earlier treatment in most cases had been begun by other doctors and the unsatisfactory results of this therapy had motivated referral of the patient to hospital. Approximately half of the patients who were treated primarily with nifuratel or with metronidazole without effect responded when the other preparation was used. In the group of patients not cured with other treatment (= local treatment of different forms) the effect of nifuratel was approximately the same as overall. Of the 4 patients who failed to respond to nifuratel 3 had some benefit from metronidazole. Among the patients reported as uncured after 2 courses of nifuratel and 1 course of metronidazole there was one who was cured after a total of 3 courses of nifuratel and one after a total of 4 courses alternating with other forms of local treatment. Two patients received metronidazole orally and nifuratel vaginal tablets twice daily for 10 days and both were cured.

No serious side effects were recorded. Seven patients who previously had completely regular menstruation began menstruating 1 week too early. Urticaria occurred in 2 cases in one of

tions are given in the opposite order. It is reasonable to assume that this response to therapy depends either on the local conditions with poor distribution of the active substances or on resistant organisms. If one accepts that the cervical canal is one of the more difficult areas for the active substances to gain access to, then one would expect a comparatively high percentage of failures in those patients with cervicitis. The results of our investigation however show that this is not so. The most likely explanation is that when both metronidazole and nifuratel have failed the organisms have developed an increased resistance. It is possible that simultaneous treatment with several preparations could prevent this development.

A fungal vaginitis was noted in 29 per cent of patients following successful treatment of trichomonas vaginitis with metronidazole (Keighley 1962) but one can presumably ignore this complication with nifuratel as this drug has a fungicidal effect. This effect is being studied by us and the results will be published later.

### Conclusion

We may presume that trichomonas vaginalis like other micro-organisms is capable of adapting itself gradually making antibiotics less effective. The results of our investigation confirm the results of other workers namely that nifuratel provides a valuable addition to the medical armamentarium, with a cure rate of 77 per cent.

### SUMMARY

A new trichomonocidal preparation, nifuratel (Polmisor<sup>2</sup>) has been evaluated in 95 patients with trichomonas vaginalis vaginitis. The treatment consisted of local application for all the patients for 10 days. 60 patients received oral tablets for 7 days and 30 patients for 10 days. The results in both groups were equally good, a cure rate of 77 per cent. Factors such as parity, the duration of symptoms, prior treatment and hormonal status did not influence the results. A co-existent cervicitis did not decrease the cure rate significantly. Simultaneous treatment of the sexual partner improved the results. Combined therapy with other medicaments may further improve the cure rates.

Table VI. *Results of Treatment with Nifuratel in Relation to Other Treatment*

Previous Therapy	Nifuratel 1 Course			Metronidazole 1 Course		
	N	Cured	Not Cured	N	Cured	Not Cured
Not cured with nifuratel				18	7	9
Not cured with metronidazole	9	4	5			
Not cured with other treatment	11	7	4	3	1	1

1. Two patients were not controlled.

2. One patient was not controlled.

them only after the second course. One patient developed nausea during treatment. No side effects were serious enough that the treatment had to be withdrawn and none of the patients considered the concomitant use of oral and local administration a serious inconvenience.

### Discussion

A long term assessment of the true effect of treatment is always confused because it is often impossible to discriminate between recurrence and reinfection. "Nonspecific vaginitis" is not included in this investigation because in such cases one often suspects that *trichomonas vaginalis* is the cause but it cannot be demonstrated. A cure rate of 77 per cent as the result of treatment with nifuratel must be judged as good. We have not been able to achieve results as good as those previously published, but the difference is not very great and could depend upon, among other things, different methods of selection and different lengths of follow up time. For example our results follow closely those shown in Table I if one compares them with those investigations where the results after the first to third month of observation are shown.

It can be seen from our investigation that if treatment with metronidazole failed, then the results with nifuratel might be expected to yield approximately a 50 per cent cure in the treated patients and the same conclusion can be drawn if the prepara-

## TREATMENT OF CANDIDAL VAGINITIS WITH NIFURATEL

BY

I. CHR. AURE AND H. GJONNAESS

Candidiasis is one of the most common causes of vaginitis and vulvitis. *Candida albicans* is commonly found but *Candida stellatoidea*, *Candida tropicalis* and *Candida krusei* occur not infrequently (Hurley and Morris 1964 Winner and Hurley 1966). Not all species of *Candida* are necessarily pathogenic and they may be present as saprophytes in the vagina. Earlier investigators reported *Candida* in 1-41.5 per cent of cases (Haberman et al. 1962 Rohrbach 1966 Puroila et al. 1967 Robinson et al. 1967). The aetiological factors in candidal vaginitis are not yet fully elucidated. However it has been observed that female diabetics, pregnant women and women on broad-spectrum antibiotics or steroids are particularly prone to infection (Jennison and Llewellyn-Jones 1957 Winner and Hurley 1966). Recently a high incidence has been reported in women taking hormonal contraceptives (Carterall 1966).

The impression that vaginitis is more often caused by *Trichomonas* than by *Candida* has changed in recent years and the reverse appears to be true in many countries (Read 1962 Rohrbach 1966). Pace and Schantz (1956) identified *Candida* seven times more often as *Trichomonas* in non-pregnant women with vaginitis and 15 times as often in pregnant women. Mixed trichomonal-candidal infection is relatively common, particularly in pregnant women (Lang et al. 1962 Alteras et al. 1965). However *Candida* may easily be overlooked in wet smears examined by direct microscopy if *Trichomonas* also is present.

### Acknowledgement

The authors wish to express their thanks to Mr. Ake Nilson, AB Bofors Nobel Pharma for valuable advice and technical help and also to AB Bofors Nobel-Pharma who have provided us with this new preparation.

### REFERENCES

- Arnold M. *Ther Umsch.* 23 356 1966  
 Aure J. Chr. and Gjonnaess H. *Acta obstet. gynec. scand.* 48 95 1969  
 De Camerl I. *Lancet I* 1042 1966  
 Chappaz G. and Bertrand P. *Gynaecologia* 160 17 1965  
 Dagenais Pérusse P., Baril E., Ouadahi S., Bener R. and Noël A. *Union Méd. Canada*, 93 1228 1964  
 Imperato S. *Igiene Mod.* 56 635 1963  
 Jennison R. F., Stenson P. and Watt L. *J. Clin. Pathol.* 14 431 1961  
 Keighley E. E. *Brit. Med. J* 11 93 1962  
 Leoni R. *Riv. Ostet. Ginec.* 19 106 1964  
 Peterson W. F., Stauch J. E. and Ryder C. D. *Amer. J. Obstet. Gynec.* 94 343 1966  
 Sagone I. *Minerva Ginec.* 17 654 1965  
 Sarnella A. *Riv. Ostet. Ginec. Prat.* 48 625 1966  
 Scuri R. *Chim. Ther.* 3 181 1966  
 Sherman A. *Lancet II* 836 1963  
 Spensden E. K. *Tidsskr. Norske Lægeforen.* 82 957 1962  
 Vartiainen E. and Widholm O. *Duodecim* 82 755 1966  
 Watt L. *Practitioner* 195 613 1965  
 Watt L. and Jennison R. F. *Brit. Med. J* 1 76 1962  
 Ødegaard K. *Proc. Northern Dermat. Soc.* 16th meeting, p. 61 1962 (a)  
 Ødegaard K. *Nord. Med.* 68 1483 1962 (b)

Received on Jan. 29 1968

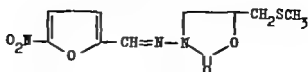
## TREATMENT OF CANDIDAL VAGINITIS WITH NIFURATEL

BY

J. CHIL. AURE AND H. GJONNAESS

Candidiasis is one of the most common causes of vaginitis and vulvitis. *Candida albicans* is commonly found but *Candida stellatoidea*, *Candida tropicalis* and *Candida krusei* occur not infrequently (Hurley and Morris 1964 Winner and Hurley 1965). Not all species of *Candida* are necessarily pathogenic and they may be present as saprophytes in the vagina. Earlier investigators reported *Candida* in 1-41.5 per cent of cases (Haberman et al. 1962 Rohatner 1966 Parola et al. 1967 Robinson et al. 1967). The aetiological factors in candidal vaginitis are not yet fully elucidated. However it has been observed that female diabetics, pregnant women and women on broad-spectrum antibiotics or steroids are particularly prone to infection (Jennison and Llywelyn-Jones 1957 Winner and Hurley 1966). Recently a high incidence has been reported in women taking hormonal contraceptives (Catterall 1966).

The impression that vaginitis is more often caused by *Trichomonas* than by *Candida* has changed in recent years and the reverse appears to be true in many countries (Reed 1962 Rohatner 1966). Pace and Schantz (1956) identified *Candida* seven times more often as *Trichomonas* in non-pregnant women with vaginitis and 15 times as often in pregnant women. Mixed trichomonal-candidal infection is relatively common, particularly in pregnant women (Lang et al. 1962 Alteras et al. 1965). However *Candida* may easily be overlooked in wet smears examined by direct microscopy if *Trichomonas* also is present.



5-[(methylthio)methyl]-3-  
[(5-nitrofurfurylidene)amino]-  
2-oxasolidinone

Fig 1 Nifuratel

In contrast to *Trichomonas* which is also found in the cervix and urethra candidiasis is usually confined to the vagina and vulva. Local treatment should therefore be effective. Various methods of treatment have been used, such as douching with alkaline solutions and painting with gentian violet resulting in cure in about 60 per cent of cases (Jennison and Livelym-Jones 1957) More recently various antibiotics such as Candicidin and Chlordantoin, have been applied locally the reported cure rates ranging from 50 to 75 per cent (Nathanson 1960 Mendel and Bone 1961 Kantor *et al.*, 1966) It has been reported that Nystatin results in cure in 60–100 per cent of cases (Pace and Schantz 1956 Charnock 1958 Kantor *et al.* 1966)

Nifuratel is a new drug, synthesized by Polichimica SAP of Milan. It is a derivative of nitrofurans, its generic name being Nifuratel It will be available as Polmiron<sup>®</sup> in the Scandinavian countries. Fig 1 shows its chemical configuration In a previous trial (Gjonnaess and Aure 1969) combined local and systematic treatment also proved to be effective against trichomoniasis. It has previously been studied in Spain by Macia and Dexeus (1966) and in Japan by Magara (1967) the former workers achieving cure in 23 out of 33 cases of candidal vaginitis and the latter in 28 of 41 cases.

#### Material and Method

Nifuratel was tested from March 1966 to May 1967 on 60 patients with candidal vaginitis In each case fungus was identified in wet

Table 1. Results of Treatment with Nifuratel in the Present Series

	1st Course			2nd Course			Cured after 2nd Course	%
	No. of Patients	Cured	Im- proved	No. of Patients	Cured	Im- proved		
Pregnant	16	11	5 <sup>a</sup>	4	2	2	13	81
Non-pregnant	44 <sup>b</sup>	35	8 <sup>c</sup>	3	1	2	36	82
Total	60 <sup>d</sup>	46	13	7	3	4	49	82

One patient did not return for follow-up examination after the 2nd course.

One patient did not return for follow-up examination.

One patient did not follow the instructions for treatment properly but her condition had improved. Two patients were well subjectively but microscopy revealed solitary mycelia. The condition of two patients had improved after the 1st course. None of these patients returned for follow-up examination after the 2nd course.

studies by direct microscopic examination. Sixteen patients were pregnant, one patient was taking hormonal contraceptives and two patients had gonorrhoea in addition to candidiasis. Three patients were menopausal when the first symptoms of the infection appeared. None of the patients had diabetes. Two vaginal tablets each of 0.25 g Nifuratel were inserted daily one in the morning and the other in the evening, for 15 days treatment was discontinued during menstruation. Follow-up examination was carried out 10 days or more after completion of treatment. Patients who were found to be free from symptoms and who had a pale healthy vaginal epithelium and normal vaginal secretion as judged both macroscopically and microscopically were considered cured. If the first course of treatment did not result in cure a second course was given following the same regime as described above. This second course was required in 13 cases. Seven patients in this latter group returned for follow-up examination after the second course of therapy. Regular follow-up examinations over a prolonged period of time were not possible because the patients lived at a great distance from the hospital but each patient was instructed to attend if she noted the slightest symptoms of recurrence of the infection.



### Results

The results of treatment are shown in Table I. Treatment was effective in 49 out of the 60 patients in this series (82 per cent). There was no difference in the response in pregnant and non-pregnant women. The patients who were not completely cured showed marked improvement, both subjectively and objectively.

Four patients had mixed trichomonal-candidal infection. They were first treated with metronidazole and thereafter with Nifuratel, the treatment resulting in cure. Of 5 patients who had been treated unsuccessfully with Nystatin, 4 were cured following a course of Nifuratel and the condition of the fifth patient, who was pregnant, improved markedly.

No side-effects were detected.

### SUMMARY

A new drug, Nifuratel (Polmiror<sup>®</sup>) was used for the treatment of vaginal candidiasis in 60 women, 16 of whom were pregnant. Two vaginal tablets each of 0.25 g Nifuratel were inserted daily, one in the morning and the other in the evening, for 14 days. Both pregnant and non-pregnant women responded equally well to this therapy. The results were as follows: 46 patients were cured after one course of treatment and a further 3 patients following a second course; the cure rate thus being 82 per cent. The condition of the other 11 patients improved markedly. The drug proved to be a valuable therapeutic agent in cases of candidal vaginitis.

### Acknowledgement

The authors are most grateful to Mr Ake Nilson, AB Bofors Nobel-Pharma, Sweden, for his advice and technical help.

### REFERENCES

- Aleras, I., Grigoriu, D., Lazăr, M., Porojan, I. and Gavrilescu, M. *Dermatologica* (Basel) 111: 309, 1965.  
Catterall, R. D. *Lancet* 2: 830, 1966.  
Charnock, F. N. *S. Afr. med. J.* 32: 556, 1959.

- Gjessum H and Aare J C. *Acta obstet. gynec. scand.* 33 85 1959
- Haberman S Mendel E B Hall III K and Ramsey L. *Obstet and Gynec.* 20 639 1962
- Harley R and Morris E D J. *Obstet. Gynaec. Brit. Cwith.* 71 692, 1964
- Jenkinson R F and Llynwalyn-Jones J D. *Brit. med. J* 2 145 1957
- Kantor H I Kamholz J H and Boulay S H. *Sch. med. J (Bham, Ala)* 59 535 1966
- Lang W R Fritz M A and Mendel E H. *Obstet. and Gynec.* 20 788, 1962
- Macie C and Dexeus S. *Rev esp Obstet. Ginec.* 25 308 1966
- Megars M. *San Fojnika no Zlasi* 16 6, 539, 1967
- Mendel E B and Bone F W. *Amer J Obstet Gynec.* 82 540 1961
- Nathanson E A. *Obstet and Gynec.* 16 601 1960
- Pace H R and Schmitt S I J. *Amer med. Ass.* 162 268 1946
- Piccola E Iekholz M and Osterlund K. *Ann. Chir. Gynaec. Fern.* 56 95 1967
- Reed J D J. *Amer med. Wom. Ass.* 17 437 1962
- Robinson S C Nicholes W C Lee D T Wendlin J M and Zwickler B. *Canad. med. Ass. J* 96 583, 1967
- Rohatner J J. *Brit. J vener. Dis.* 42 197 1966
- Wawer H J and Harley R. *Symposium on Candida Infections. Edinburgh and London, 1966*

Received on Jan. 29 1968

## ULTRASTRUCTURE OF HUMAN CHORIOCARCINOMA

BY

M. KNOTH H. HESSELD AHL AND J. FALCK LARSEN

The ultrastructure of normal human trophoblast has been described by several investigators (Larsen 1963). However observations on malignant trophoblast are few because chorioncarcinoma is a rare tumour. Wakitani (1962) dealing with one patient obtained material by hysterectomy and from metastases. Onoé (1962) has a single illustration showing characteristic features of choriocarcinoma in a review on the electron microscopy of human malignant growths. Because of the lack of clinical material, transplantable tumour carried in the hamster cheek pouch has been studied by Wynn and Davies (1964) and in hamster liver by Larsen *et al* (1967).

During the latter study many strange phenomena were observed which had not been described in previous papers on human biopsies. When the opportunity presented biopsies were obtained by us from two patients with choriocarcinoma and this tissue was compared with the transplantable tumour.

### *Material and Methods*

Tissue was obtained from two patients with choriocarcinoma and from 26 hamsters with transplanted human choriocarcinoma in serial cheek pouch passage.

The tissue from the patients was obtained and treated as follows.

Case one: A curettage performed in a 32 year-old woman revealed a hydatidi

form mole. Spotting recurred, and two months later a second curettage was carried out. Choriocarcinoma was suspected. The diagnosis was confirmed by hysterectomy. Two weeks later metastases were observed in the vagina and biopsies were obtained for electron microscopy. The tissue was fixed in osmic tetroxide and embedded in Durcupan®. The patient was treated with Methotrexate® and has now survived for two years. Chorionic gonadotropin (HCG) has not been detected in the urine since chemotherapy was completed.

*Case two.* A 29-year-old woman was admitted to the Municipal Hospital in Copenhagen (Kommunehospitalet) because of vaginal bleeding during the fifth month of pregnancy. The haemorrhage became excessive and as the cervix remained closed, the uterine cavity was emptied by abdominal hysterotomy. A hydatodeform mole was found and removed. As the patient continued to excrete chorionic gonadotropin for six months hysterectomy was performed. A small tumour was found in the fundus, and microscopic examination revealed that it consisted of cytotrophoblast. Cytotrophoblast was also found in the paratretal venosa. Tissue was fixed for electron microscopy in osmic tetroxide and embedded in Vestopal®. The patient was treated with four courses of Methotrexate® but suffered severe pain, became emaciated and died. No autopsy was performed.

*The transplanted tumour.* This tumour was obtained during experiments in which the Greene choriocarcinoma (Hertz 1961) was carried by hamster cheek pouch transfer approximately every ten days. The experiments were done to study the relation between tumour and uninvaded tissue (Lerner et al. 1967). The tumour was fixed in osmic tetroxide and embedded in Epon®.

The ultrathin sections were cut on Porter Blum and LKB-ultramicrotomes, stained with lead citrate and uranyl acetate, and examined in a JEOL JEM-6 C electron microscope.

## Results

### *Case one Compared with the Transplanted Tumour*

The fine structure of the choriocarcinoma from the first patient and from the cheek pouch transplanted Greene tumour was identical. Both consisted mainly of two types of cells. One type (Fig. 2) resembled the cytotrophoblast of the early human placenta while the other type was a giant cell with many characteristics of the normal syncytiotrophoblast (Figs. 3 and 5).

The first type of cell, the cytotrophoblast, was regular in shape usually elongated or spindle-shaped. The nucleus was large with an irregular nuclear membrane often with infoldings (Fig. 2). The nucleolus was skein-like and rather large. The cell membrane

was regular with few indentations but in some places microvilli of variable form and amount were found. They were often short and clubbed and sometimes branching. In this type of cell very limited endoplasmic reticulum could be found. The RNA was present in the form of clusters of ribosomes lying free in the cytoplasm. However in some cells the ribosomes were found as part of the sparse endoplasmic reticulum (Fig. 6). The golgi apparatus was abundant in many cells. In some a large part of the cytoplasm was occupied by accumulations of smooth golgi membranes and vesicles (Fig. 7). The mitochondria measuring about 0.5 to 3  $\mu$  appeared slightly larger than those of normal trophoblast. They were numerous many had a polygonal form with few curled cristae and abundant matrix of medium electron density (Figs. 2 and 8) but mitochondria of more ordinary shape were also found (Fig. 8).

The cytoplasm contained variable amounts of agranular reticulum and vacuoles (Fig. 2) some containing fine granular substance. Occasionally centrosomes were found (Fig. 7).

The other cell type the giant cell (Figs. 3 and 4) measured more than 100  $\mu$  in diameter. The nucleus was in a central position in most of the cells. The nuclear membrane (Fig. 9) was very irregular with deeper infoldings than those of the cytotrophoblastic cell type (Fig. 2). Many of the nuclear protrusions were branching and the nuclear pores were rather large up to 1000 Å in diameter (Fig. 9). This cell type was identified as the multinucleated type in the light microscopic sections but only occasionally was more than one nucleus found in the ultrathin sections. The cell membrane often had more slender microvilli than those found on the surface of the cytotrophoblast. At the base of the microvilli small vesicles indicated pinocytotic activity (Fig. 3). The endoplasmic reticulum of this type of cell was of the rough-surfaced type and consisted of numerous short channels or vacuoles (Figs. 3 and 4). The channels were often found to be slightly dilated and somewhat irregular in outline. Polyribosomes free in the cytoplasm were also found. In some cells endoplasmic reticulum of the common type was observed (Fig. 9). The mitochondria of the giant cells (Figs. 3 and 4) were relatively smaller and less numerous and they were more regular in shape.

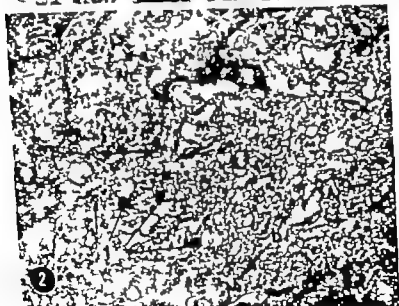


Fig 1 Portion of cytotrophoblastic cell from case two. Many cub shaped microvilli on the surface (MV). The mitochondria (M) have few cristae. In the cytoplasm large inclusions (I) are found. NC, Nucleus.  $\times 12,500$ .

Fig 2 Portions of two cytotrophoblastic cells from the transplanted tumour. The nucleus (NC) is very irregular. The mitochondria (M) are large irregular in shape and have few cristae. Inclusions (I) are numerous. The cells are connected by desmosomes (D).  $\times 8000$ .

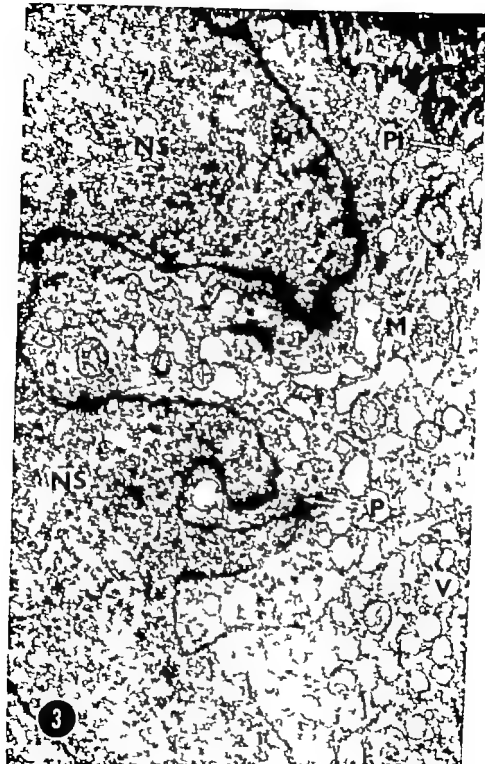


Fig. 3 Portion of syncytial type of tumour cell from transplanted tumour. The nucleus (NS) is very large with nucleolus. The cytoplasm contains many vacuoles (V). Some of the ribosomes are attached to these vacuoles. P- Nuclear pores, M- Mitochondria. The cell membrane possesses microvilli.



Fig 4 Giant cell from case one. The mitochondria (M) have few cristae  
 NS Nucleus. The cytoplasm is rich in golgi membranes (G) MY Myelin  
 Figure. L Lipid granule. LY Lysosomes ER Endoplasmic reticulum  $\times 28,000$   
 Fig 5 Syncytiotrophoblast of transplanted tumour containing lactosomes (LA)  
 NS Nucleus of syncytiotrophoblast NC Nucleus of cytotrophoblast  $\times 2000$



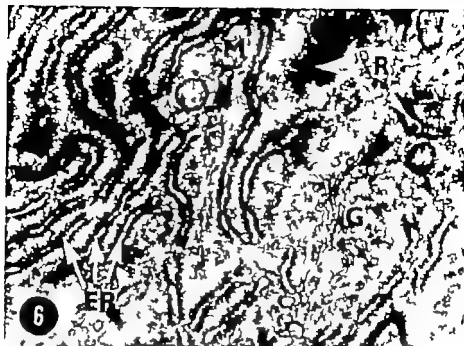


Fig 6 Portion of cytotrophoblastic cell from case one. Large accumulation of endoplasmic reticulum (ER). G Golgi apparatus. M Mitochondrion. R. Ribosomes.  $\times 32,000$

Fig 7 Portion of cytotrophoblastic cell from case one. M Mitochondrion. AR. Agranular reticulum. G Golgi apparatus. ER. Endoplasmic reticulum. C. Centrosomes. MI. Mitochondrion of more normal shape. F Fibrils of intercellular substance.  $\times 28,000$

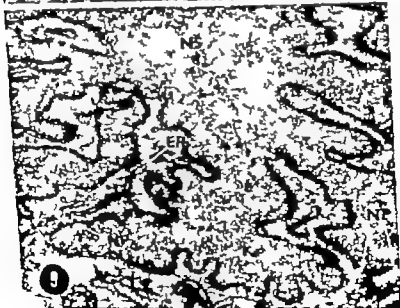


Fig. 8 Mitochondria from cytotrophoblast of transplanted tumour. M. Large, star shaped mitochondrion with few cristae. ML. Mitochondrion of ordinary type  $\times 18,000$

Fig. 9 Portion of syncytotrophoblast from case one. The nucleus (NS) has very deep infoldings. The nuclear pores (NP) are large. ER. Endoplasmic

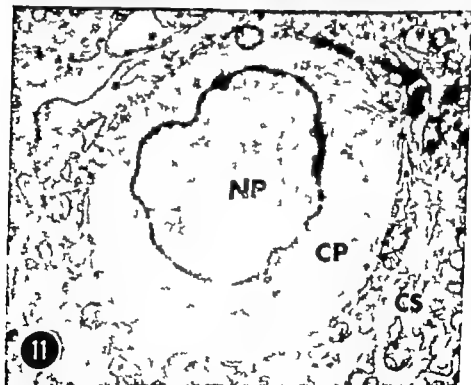


Fig. 10 Syncytiotrophoblast from transplanted tumor exhibiting phagocytosis. NL Nucleus of phagocytosed liver cell. In complete membranes a found between cytoplasm of liver cell (CL) and cytoplasm of trophoblast (CS) NS Nucleus of trophoblast LY Lysosomes  $\times 10,000$

Fig. 11 Ghost cell from case one NP Nucleus of phagocytosed cell Membrane between the phagocytosed cell and the cytoplasm of the trophoblast (CS) is broken in many places (White arrow) CP Cytoplasm of phagocytosed cell.  $\times 11,000$

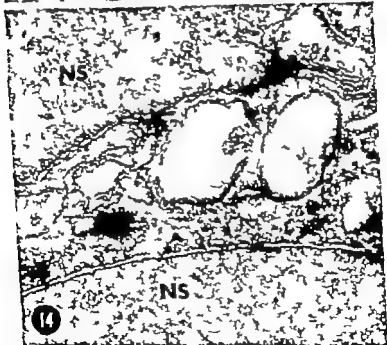


Fig. 12. Myelin figure (M) and mitochondrial like figure (White arrow) inside the nucleus of tumour cell from case one. Black arrows indicates nuclear membrane  $\times 22,000$

Fig. 13. Unidentified structures in the cytoplasm of trophoblastic giant cell of case one. In some of these the internal structure resembles that of desmosomes (White arrows)  $\times 70,000$

Fig. 14. Intracellular desmosome in giant cell from case one. NS. Nucleus. D. Desmosome.  $\times 38,000$

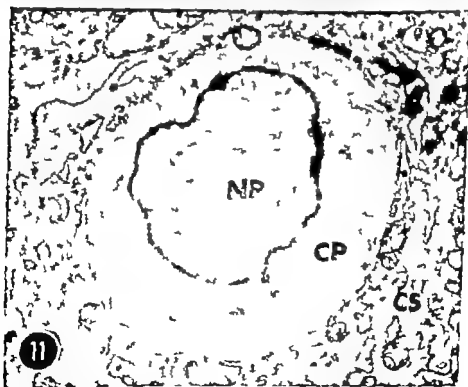


Fig 10. Syncytiotrophoblast from transplanted tumour exhibiting phagocytosis. NL. Nucleus of phagocytosed liver cell. Incomplete membranes are found between cytoplasm of liver cell (CL) and cytoplasm of trophoblast (CS). NS. Nucleus of trophoblast. LY. Lysosomes.  $\times 10,000$ .

Fig 11. "Ghost cell" from case one. NP. Nucleus of phagocytosed cell. Membrane between the phagocytosed cell and the cytoplasm of the trophoblast (CS) is broken in many places (White arrows). CP. Cytoplasm of phagocytosed cell.  $\times 11,000$ .

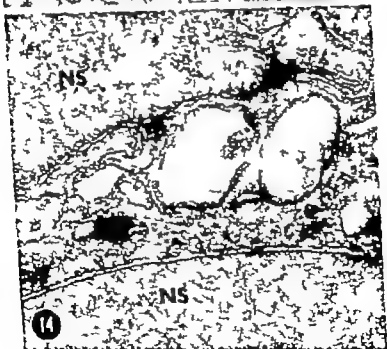


Fig. 12. Myelin figure (M) and mitochondrion-like figure (White arrow) inside the nucleus of tumour cell from case one. Black arrows indicate nuclear membrane  $\times 22,000$ .

Fig. 13. Unidentified structures in the cytoplasm of trophoblast giant cell of case one. In some of these the internal structure resembles that of desmosomes (White arrows)  $\times 70,000$ .

Fig. 14. Intracellular desmosome in giant cell from case one. NS: Nucleus. D: Desmosome  $\times 38,000$ .

with more cristae than those described in the cytotrophoblastic cells. In many cells lysosomes and myelin figures were observed (Fig 4)

Some giant cells had additional ultrastructural features. Intracellular irregular canaliculi or lacunae were found in some of the giant cells (Fig 5) The spaces were lined with microvilli, and contained an agranular substance of low electron density

Phagocytic activity was seen in several giant cells (Fig 10) The cytoplasm was more or less filled with partly digested cellular material. Sometimes this material was surrounded by a shadow like membrane retaining some of the characteristics of a phagocytosed nucleus (Fig 11) In other regions of the cytoplasm collections of amorphous material of medium electron density with incomplete surrounding membranes were seen (Fig 1)

In many areas bundles of collagen with characteristic periodicity were observed. In other areas the intercellular substance was rich in fibrillar material of high electron density almost completely surrounding the tumour cell. Sometimes the fibrils were condensed close to the cell membrane simulating a basement membrane.

In some of the cells intranuclear bodies were observed. One of these was a structure resembling a myelin figure surrounded by a condensation in the nuclear substance and in the same nucleus a mitochondrion like structure was also found (Fig 12) In other nuclei the intranuclear body consisted of a granular substance surrounded by a membrane.

Desmosomes were observed between both types of tumour cells. In the transplanted tumour desmosomes were found also between the tumour cells and the host tissue (Larsen *et al* 1967) In one of the cells from case one was found an intracellular desmosome (Fig 14) In other cells from the same tumour the cytoplasm contained unidentified desmosome-like figures (Fig 13) Desmosomes connecting tumour cells with host cells were not convincingly shown in the tissue from either case one or case two

Almost all cells could be classified as cytotrophoblastic or giant cells (syncytiotrophoblast) However a few cells had some of the characteristics of both and may represent a transitional

type. Other cells differed by having an extraordinarily high content of golgi elements (Fig. 10 in *Larsen et al.* 1967).

**Case two** The tissue obtained from the second patient consisted of the cytotrophoblastic type only. This type (Fig. 1) was similar to the corresponding element in the tumour from case one and in the transplanted tumour. The mitochondria had the same simple appearance with few cristae. Almost all the cells had numerous microvilli. Between the microvilli vacuoles beneath the cell membrane indicated pinocytotic activity. The cytoplasm was rich in large inclusions (Fig. 1) consisting of fine granular substance bordered by an incomplete membrane.

### Discussion

The biopsies from case one showed the typical plexiform pattern of trophoblast, and the admixture of blood clot and fragments of necrotic decidua, diagnostic of choriocarcinoma (*Herrig and Mansell*, 1956).

*Benirschke and Driscoll* (1967) stated that choriocarcinoma is always composed of both elements of trophoblast syncytium and cytotrophoblast. Only cytotrophoblast was found in the sparse material from the second patient, and there was no opportunity to obtain additional biopsies. *Novak and Seth* (1954) also found both elements in the examination of biopsies from 74 cases registered in the Mathison choriocarcinoma registry but in one case a great preponderance of cytotrophoblastic cells was clearly evident. In our second case the clinical history leaves no doubt that the patient died from choriocarcinoma. Moreover the ultrastructure of the cytotrophoblast from that patient was very similar to that of the cytotrophoblastic cells of case one and the transplanted tumour.

The similarity in the ultrastructure of transplanted tumour and tissue obtained from the patients confirms on the submicroscopic level the observations by *Hertz* (1961) who found no change in the morphology of choriocarcinoma during its transfer from man to experimental animals.

In many ways the ultrastructure of tumour resembled that of normal placental trophoblast. The majority of cells could be



classified into two groups according to their resemblance to either cytotrophoblast or syncytiotrophoblast of the normal placental villus.

The first type differed from normal first trimester cytotrophoblast primarily in the size of the cell and in the irregularity of the nucleus and mitochondria. The cytotrophoblast of the human placenta measures about  $20 \times 50 \mu$  but the malignant cytotrophoblast often measures  $100 \mu$  or more in diameter. The malignant nuclei are larger and the nuclear membrane is more irregular.

The second type the giant cell resembled the syncytiotrophoblast of the early normal placenta (Boyd and Hughes 1954 Wislocki and Dempsey 1955 Terakis 1963 Lister 1964 Anderson and McKay 1966), but differed in the appearance of the nuclei. These were much larger with complicated infoldings. The nuclear pores measured about  $1000 \text{ \AA}$ , which is about twice the normal size (Freeman 1964 Fawcett 1966).

No single morphological characteristic is specific of cancer cells but many features found in choriocarcinoma are similar to those observed in other types of malignant cells. Two of these features are nuclear hypertrophy and irregularity. The deep indentations of the nuclear membrane increase the surface of the nucleus. Therefore the possibility of facilitating nucleo-cytoplasmic interchanges must be considered. The large size of the nuclear pores may have the same consequence. Although rare nuclei with irregular nuclear membranes can sometimes be found in the normal syncytiotrophoblast (Wislocki and Dempsey 1955).

Cancer cells generally have a simpler ultrastructure than fully differentiated normal cells (Oberling and Bernhard 1961). In our study however the tumour cells retained many of the cytoplasmic characteristics of the normal trophoblast.

The golgi apparatus was prominent in some of the cells, especially in those of the cytotrophoblastic type. A prominent golgi apparatus has been found in mouse mammary tumours (Bernhard and Guérin 1957) but generally golgi elements are not conspicuous in malignant cells (Oberling and Bernhard 1961). The mitochondria were irregular in shape with few curled cristae often having a swollen appearance. This type of mitochondrion

was found to be the feature which usually distinguished the tumour cells from normal trophoblast. This swollen type of mitochondria with few cristae has been described in many types of tumour (Oberling and Bernhard 1961). Unsatisfactory fixation cannot explain this appearance of the mitochondria as other cell organelles were adequately fixed, and normal mitochondria were also found in the connective tissue and liver cells in the same sections.

The amount of endoplasmic reticulum in cancer cells tends to decrease with dedifferentiation of the tumour cell (Oberling and Bernhard 1961). Only sparse lamellae can be observed and most of the RNA-granules are found lying free in the cytoplasm. This picture is seen in the majority of cytotrophoblastic cells in all the investigated choriocarcinoma (Midgley and Pierce 1963, Wyne 1963). The syncytiotrophoblastic cells on the other hand, could be recognized by a cytoplasm rich in endoplasmic reticulum which is characteristic of syncytial trophoblast of the normal placenta. This type of endoplasmic reticulum consists of dilated sacs or vacuoles with a homogeneous content of medium dense material.

Much effort has been made to define the term choriocarcinoma. The clinician would prefer a classification of trophoblastic growths indicating the prognosis. In most cases, patients without chemotherapy die within two years. However Brewer *et al.* (1961) reported that of 147 patients noted in the Mathieu register as cases of choriocarcinoma treated by surgery and supplemented in two instances by irradiation, 21 survived more than five years. The tissue from these 21 patients was studied carefully by the authors as well as by Hertig (1961) but they could not point out any morphological criteria which could separate the 15 per cent of curable lesions from the 85 per cent which killed the patients despite treatment.

Due to this lack of light microscopic criteria it would be of great value if a study of the ultrastructure could give clues to the prognosis. The degree of cytoplasmic simplicity and the mitochondrial deformity may be indicative of increasing malignancy. Only a submicroscopic examination of biopsies from many patients with choriocarcinoma and a clinical follow-up study might show

classified into two groups according to their resemblance to either cytotrophoblast or syncytiotrophoblast of the normal placental villus.

The first type differed from normal first trimester cytotrophoblast primarily in the size of the cell and in the irregularity of the nucleus and mitochondria. The cytotrophoblast of the human placenta measures about  $20 \times 50 \mu$  but the malignant cytotrophoblast often measures  $100 \mu$  or more in diameter. The malignant nuclei are larger and the nuclear membrane is more irregular.

The second type the giant cell resembled the syncytiotrophoblast of the early normal placenta (Boyd and Hughes 1954 Wislocki and Dempsey 1955 Terrakis 1963 Lister 1964 Anderson and McKay 1966) but differed in the appearance of the nuclei. These were much larger with complicated infoldings. The nuclear pores measured about  $1000 \text{ \AA}$  which is about twice the normal size (Freeman 1964 Fawcett 1966).

No single morphological characteristic is specific of cancer cells but many features found in choriocarcinoma are similar to those observed in other types of malignant cells. Two of these features are nuclear hypertrophy and irregularity. The deep indentations of the nuclear membrane increase the surface of the nucleus. Therefore the possibility of facilitating nucleo-cytoplasmic interchanges must be considered. The large size of the nuclear pores may have the same consequence. Although rare nuclei with irregular nuclear membranes can sometimes be found in the normal syncytiotrophoblast (Wislocki and Dempsey 1955).

Cancer cells generally have a simpler ultrastructure than fully differentiated normal cells (Oberling and Bernhard 1961). In our study however the tumour cells retained many of the cytoplasmic characteristics of the normal trophoblast.

The golgi apparatus was prominent in some of the cells especially in those of the cytotrophoblastic type. A prominent golgi apparatus has been found in mouse mammary tumours (Bernhard and Guérin 1957) but generally golgi elements are not conspicuous in malignant cells (Oberling and Bernhard 1961). The mitochondria were irregular in shape with few curled cristae often having a swollen appearance. This type of mitochondrion

if these criteria can be used. However this would be difficult because of the rarity of the disease in most parts of the world. Furthermore it may not be possible to use such criteria even with electron microscopy if the variations in clinical course are caused by host differences rather than by variations in tumour malignancy.

Choriocarcinoma produces gonadotrophic hormones. Using the fluorescent antibody technique evidence has been presented favouring the theory that HCG is produced in the syncytiotrophoblast (Midgley and Plörce 1962). This view is supported by the presence of abundant endoplasmic reticulum in the syncytiotrophoblast in contrast to the sparse amount in the cytotrophoblast.

It is difficult to distinguish HCG biologically and chemically from the luteinizing hormone (LH) of the pituitary. Farquhar and Rinehart (1954) in their studies of the rat pituitary found that castration resulted in the proliferation of signet ring cells with a large cytoplasmic vacuole and also of cells with a filigree-like cytoplasm due to innumerable irregular vacuoles. Basing their arguments on bio assays, these authors suggested that the former type of cell is the source of follicle stimulating hormone while the later is the source of luteinizing hormone. It is interesting that cells similar to both types have been found in the choriocarcinoma transplanted to hamster liver (Larsen *et al.* 1967).

Electron dense granules of the kind found in the pituitary have not been observed in the tumour nor are such granules found in the normal placental trophoblast. However in some of the tumour cells of both types fine granular material of medium electron density was seen sometimes enclosed within a membrane. This material is well known from studies on the cytoplasm of early placental syncytiotrophoblast (Terzakis 1963) and may represent the ultrastructural picture of HCG.

It is well known that viruses may cause leukaemia and other malignant growths. Because of the unknown aetiology of choriocarcinoma this tissue was examined to detect possible evidence of virus. No virus like particles or crystals were found. However virus affected tissue may not show any specific changes. The cell injuries may only include enlargement of the nuclei nucleolar

if these criteria can be used. However, this would be difficult because of the rarity of the disease in most parts of the world. Furthermore, it may not be possible to use such criteria even with electron microscopy if the variations in clinical course are caused by host differences rather than by variations in tumour malignancy.

Choriocarcinoma produces gonadotrophic hormones. Using the fluorescent antibody technique, evidence has been presented favouring the theory that HCG is produced in the syncytiotrophoblast (Midgley and Pierce 1962). This view is supported by the presence of abundant endoplasmic reticulum in the syncytiotrophoblast in contrast to the sparse amount in the cytotrophoblast.

It is difficult to distinguish HCG biologically and chemically from the luteinizing hormone (LH) of the pituitary. Farquhar and Rinehart (1954) in their studies of the rat pituitary found that castration resulted in the proliferation of signet ring cells with a large cytoplasmic vacuole and also of cells with a filigree-like cytoplasm due to innumerable irregular vacuoles. Basing their arguments on bio-assays, these authors suggested that the former type of cell is the source of follicle stimulating hormone while the latter is the source of luteinizing hormone. It is interesting that cells similar to both types have been found in the choriocarcinoma transplanted to hamster liver (Larsen *et al.* 1967).

Electron dense granules of the kind found in the pituitary have not been observed in the tumour nor are such granules found in the normal placental trophoblast. However, in some of the tumour cells of both types fine granular material of medium electron density was seen, sometimes enclosed within a membrane. This material is well known from studies on the cytoplasm of early placental syncytiotrophoblast (Terzakis 1963) and may represent the ultrastructural picture of HCG.

It is well known that viruses may cause leukaemia and other malignant growths. Because of the unknown aetiology of choriocarcinoma, this tissue was examined to detect possible evidence of virus. No virus-like particles or crystals were found. However, virus affected tissue may not show any specific changes. The cell injuries may only include enlargement of the nuclei, nucleolar

if these criteria can be used. However this would be difficult because of the rarity of the disease in most parts of the world. Furthermore it may not be possible to use such criteria even with electron microscopy if the variations in clinical course are caused by host differences rather than by variations in tumour malignancy.

Choriocarcinoma produces gonadotrophic hormones. Using the fluorescent antibody technique evidence has been presented favouring the theory that HCG is produced in the syncytiotrophoblast (Midgley and Pierce 1962). This view is supported by the presence of abundant endoplasmic reticulum in the syncytiotrophoblast in contrast to the sparse amount in the cytotrophoblast.

It is difficult to distinguish HCG biologically and chemically from the luteinizing hormone (LH) of the pituitary. Farquhar and Rinehart (1954) in their studies of the rat pituitary found that castration resulted in the proliferation of signet ring cells with a large cytoplasmic vacuole and also of cells with a filigree-like cytoplasm due to innumerable irregular vacuoles. Basing their arguments on bio-assays these authors suggested that the former type of cell is the source of follicle stimulating hormone while the latter is the source of luteinizing hormone. It is interesting that cells similar to both types have been found in the choriocarcinoma transplanted to hamster liver (Larsen *et al.* 1967).

Electron dense granules of the kind found in the pituitary have not been observed in the tumour nor are such granules found in the normal placental trophoblast. However in some of the tumour cells of both types fine granular material of medium electron density was seen sometimes enclosed within a membrane. This material is well known from studies on the cytoplasm of early placental syncytiotrophoblast (Terzakis 1963) and may represent the ultrastructural picture of HCG.

It is well known that viruses may cause leukaemia and other malignant growths. Because of the unknown aetiology of choriocarcinoma this tissue was examined to detect possible evidence of virus. No virus like particles or crystals were found. However virus affected tissue may not show any specific changes. The cell injuries may only include enlargement of the nuclei nucleolar

if these criteria can be used. However this would be difficult because of the rarity of the disease in most parts of the world. Furthermore it may not be possible to use such criteria even with electron microscopy if the variations in clinical course are caused by host differences rather than by variations in tumour malignancy.

Choriocarcinoma produces gonadotrophic hormones. Using the fluorescent antibody technique evidence has been presented favouring the theory that HCG is produced in the syncytiotrophoblast (Midgley and Pierce 1962). This view is supported by the presence of abundant endoplasmic reticulum in the syncytiotrophoblast in contrast to the sparse amount in the cytotrophoblast.

It is difficult to distinguish HCG biologically and chemically from the luteinizing hormone (LH) of the pituitary. Farquhar and Rinehart (1954) in their studies of the rat pituitary found that castration resulted in the proliferation of signet ring cells with a large cytoplasmic vacuole and also of cells with a filigree-like cytoplasm due to innumerable irregular vacuoles. Basing their arguments on bio-assays these authors suggested that the former type of cell is the source of follicle stimulating hormone while the latter is the source of luteinizing hormone. It is interesting that cells similar to both types have been found in the choriocarcinoma transplanted to hamster liver (Larsen *et al.* 1967).

Electron dense granules of the kind found in the pituitary have not been observed in the tumour nor are such granules found in the normal placental trophoblast. However in some of the tumour cells of both types fine granular material of medium electron density was seen sometimes enclosed within a membrane. This material is well known from studies on the cytoplasm of early placental syncytiotrophoblast (Terzakis 1963) and may represent the ultrastructural picture of HCG.

It is well known that viruses may cause leukaemia and other malignant growths. Because of the unknown aetiology of choriocarcinoma this tissue was examined to detect possible evidence of virus. No virus like particles or crystals were found. However virus-affected tissue may not show any specific changes. The cell injuries may only include enlargement of the nuclei, nucleolar

found phagocytosis in some areas especially in fields with considerable destruction of tissue and some of the phagocytized cells were blood cells. The limitation of phagocytosis to a few fields could be explained by the assumption that choriocarcinoma cells phagocytosed devitalized material only. The devitalization could have resulted from lack of blood supply or by toxic agents from the invading tumour.

In a previous publication (Larsen *et al.* 1967) desmosomes between choriocarcinoma and the invaded tissue were described. The presence of desmosomes between trophoblast and liver cells is a strange phenomenon. The cells they connect are not only from different organ systems but also from different species man and hamster.

Another strange observation is the presence of intracellular desmosome-like figures found either as small groups (Fig. 13) or interposed between two different nuclei in one cell (Fig. 14). Onoé (1962) has in a study of the ultrastructure of human choriocarcinoma a single micrograph showing intracellular desmosome-like structures. Recently Okudaira and Strauss (1967) described aggregations of similar structures in choriodenoma destruens. If these elements are desmosomes they may originate from disintegrating cell membranes between cytotrophoblastic cells as they merge into a syncytium or from the membranes of phagocytized cells.

## SUMMARY

Tumour tissue from two patients with choriocarcinoma and tumours transplanted into hamster liver were studied with the electron microscope. The ultrastructure of choriocarcinoma is compared with that of other cancer cells and with non-malignant trophoblastic tissue.

Two types of trophoblastic tumour cells were observed. The first type resembled the cytotrophoblast of normal placenta but the cells were larger and contained large irregular mitochondria with few cristae. The second type resembled the normal syncytiotrophoblast, but differed in the large irregular nuclei.

The phagocytic activity the possible ultrastructural picture of



chorionic gonadotrophin and the existence of intracellular spaces lined with microvilli are discussed. In some of the cells, nuclear inclusions and intracytoplasmic desmosome-like figures were observed. As to the aetiology of choriocarcinoma, no conclusive evidence of viral origin could be demonstrated.

### *Addendum*

Since this manuscript has been submitted, *Inferrence et al.* (Arch. Obstet. & Gynec. 72: 707, 1967) have published a paper on the ultrastructure and histochemistry of choriocarcinoma. They found an intermediate type and an extremely undifferentiated type of cell besides the cytotrophoblast and syncytiotrophoblast. The description of the ultrastructure is in good agreement with that presented in this paper. These authors describe numerous osmiophilic granules in the syncytium and they consider these to be a possible morphological expression of the secretion of chorionic gonadotropins.

### *Acknowledgements*

We thank Dr Robert L. Ehrmann, Department of Pathology Parkway Division of the Boston Hospital for Women, Brookline, Massachusetts U.S.A. for permission to use material compiled for earlier studies of the transplanted choriocarcinoma.

### REFERENCES

- Anderson W R and McKay D G. Amer J Obst & Gynec 95: 1134, 1966  
Beurichler K and Driscoll S G. The pathology of the human placenta. In: Handbuch der speziellen pathologischen Anatomie und Histologie. Vol. VII Part V ed by E. Uehlinger. Springer Verlag, Berlin, 1967 p. 494  
Bernhard H and Guern M. Proc. 2nd Int. symposium mammary carcinoma. Perugia, 1957 p. 623  
Boutelle M, Kalifat S R and Delorme J J. Ultrastruct. Res. 19: 474, 1967  
Brumer J I, Ruckhart J J and Dunbar R W. Amer J Obst & Gynec 81: 574, 1961  
Boyd, J D and Hughes A W. J Anat. Lond. 88: 356, 1954  
Farquhar J G and Ruckhart J F. Endocrinology 54: 516, 1954  
Anat. Record 121: 394, 1955  
Fauett D W. The cell, W B Saunders Company Philadelphia and London, 1966, p. 34

- Freeman J. A. Cellular fine structure McGraw Hill Book Company New York-Toronto-London, 1964 p. 85
- Hertig, A. T. Amer J Obst. & Gynec. 81 580 1961
- Hertig, A. T. and Mansell H. The tumors of the female sex organs Part one hydatidiform mole and choriocarcinoma. Armed Forces Inst. of pathol., Washington D C 1956 p. 10
- Hertz R. Human trophoblast growth evolution and identification *in vitro* and in animals. In Transcript of the first Rochester trophoblast conference Univ of Rochester NY 1961 p. 97-107
- Knoth M. Ultrastructure of the trophoblast from a normal 4 somites embryo J Ultrastr. Res. in press
- Larsen J. F. Electron microscopy of the human placenta. In. Transcript of the second Rochester trophoblast conference Univ of Rochester NY 1963 p. 280-300
- Larsen J. F. Ehrmann R. L. and Biering, F. Amer J Obst. & Gynec. 99 1109 1957
- Lister U. M. J Obst. & Gynec. Brit. Cwlt. 71 21 1964
- Midgley A. R. and Pierce G. B. J Exper. Med. 115 289 1962
- In. Transcript of the second Rochester trophoblast conference Univ of Rochester NY 1963 42-58
- Oberling, C. and Bernhard W. The morphology of the cancer cell. In. Brachet, J. and Mirsky A. E. (eds.) The Cell - Vol. V Part II. Academic Press New York and London 1961
- Okudaira Y. and Strauss L. Obst. & Gynec. 30 172, 1967
- Onof T. J. Elec. Microsc. 11 70 1962
- Terrakis J. A. J. Ultrastr. Research 9 268 1963
- Waldman T. M. J. med. Journal 12 43 1967
- Wislocki G. ■ and Dempsey E. W. Anat. Record 123 133 1955
- Wynn R. M. In. Transcript of the second Rochester trophoblast conference. Univ of Rochester NY 1963 p. 58-91
- Wynn R. M. and Davies J. Amer J Obst. & Gynec. 88 618 1964

Received on Dec. 23 1967

## PERCUTANEOUS CATHETERIZATION OF THE HYPOGASTRIC ARTERY IN LOCAL INFUSION THERAPY WITH CYTOSTATICS

BY

INGMAR FERNSTRÖM, KARL-GUSTAV MELIN AND NILS WIKQVIST

In diagnostic pelvic arteriography using compression of the femoral artery there is no need for selective catheterization of the hypogastric arteries. However local infusion therapy with cytostatics in cases of advanced cervical carcinoma or malignant trophoblastic disease requires introduction of thin catheters into the hypogastric arteries. It is advantageous for many reasons if these catheters can be introduced without subjecting the patient to distressing surgical procedures. It is also necessary that the catheters be maintained in proper position during a comparatively long period of time, with a minimum of discomfort for the patient and without causing complications.

Some authors have introduced the catheters by extraperitoneal surgical exposure of the hypogastric arteries (Cahill and Zett 1961 Hulka and Biesel 1965 Sullivan *et al.*, 1960 Trussel and Milford Barberson 1961) whereas others have introduced the tubes in the gluteal arteries (Laufer). Several investigators have given local infusion of methotrexate after percutaneous puncture of the femoral artery and introduction of a catheter into the lower aorta (Baseman *et al.* 1966 Botman *et al.* 1956 Krakoff and Sullivan 1958). The first two methods mentioned have the drawback of necessitating a major surgical procedure, whereas

the last distributes the cytostatic agent into both the external and internal iliac arteries. It would be of advantage if thin polyethylene catheters could be introduced into the hypogastric arteries by the percutaneous technique in the same way as in selective diagnostic arteriography of the renal, coeliac and mesenteric arteries. In these roentgen examinations the catheter is introduced percutaneously and the tip manoeuvred into position under roentgen television control. This technique for catheterization of the hypogastric artery has so far been used to a very small extent. Michaelsson 1965 described a new method by which large branches of the aorta could be catheterized easily. After some modification this technique could also be used for catheterization of the hypogastric artery. However the large diameter of the catheter (PE 240) precluded its retention in the artery for the desirable period of time. Therefore in a few cases a thin teflon catheter was introduced via the large polyethylene catheter which was subsequently withdrawn. Wholey and Jackman 1966 reported on a technique by which the catheter could be manoeuvred into the hypogastric artery by a special instrument. However the catheters used had a rather large diameter of more than 2 mm. Nilsson 1967 published a report of 30 patients in whom one or both hypogastric arteries had been catheterized by an Ödman catheter with bent tip and with an external diameter of 2.2 mm. These techniques have been used for the diagnosis of urinary bladder tumours but tubes of such large diameter and stiffness can hardly be left in the femoral and hypogastric arteries over the period of 7-10 days which is considered as a minimum for infusion of cytostatics.

A method for percutaneous catheterization of the hypogastric artery was recently described by Onnäs *et al* 1967. These authors suggested the use of Ödman catheters with a small diameter for selective catheterization of the hypogastric arteries. However even the thinnest catheters of this type are comparatively stiff and have an outer diameter of 1.8 mm.

During 1965 in a few cases we used Michaelsson's method to introduce thin catheters. However the technique was found to be difficult and time consuming and often failed. In 1966 a new method was introduced which was easy and reliable.

### Technique

Introduction of the catheter was made according to the Seldinger technique (Seldinger 1953) Needle no. PE 160 guide no 100 and polyethylene catheter no PE 100 were used. This catheter has an outer diameter of 1.52, an inner diameter of 0.86 and a length of 500 mm. In preparing the catheter the tip is tapered and the other end formed into a collar in the usual way. In addition, the tip of the tubing is heated by steam and bent into a loop as shown in Fig. 1.

Fourteen different roentgen-television combinations were tested for this purpose but most of these were unsatisfactory since the catheter could not be adequately visualized. The roentgen-television equipment finally chosen has high gain combined with good resolution of fine details. This television apparatus is equipped with a vidicon tube which has longer persistence than an orthicon or a plumbicon tube. However we have not encountered any difficulties in following the catheter tip during the manipulations.

To improve the picture quality the technique of geometric magnification was used during the catheterization. With the image intensifier input screen placed at a distance of about 50 cm from the patient a linear magnification of 2:1 is achieved. This has two advantages. In the first place image resolution is substantially increased, since the ability of the roentgen-television equipment to display small details is primarily determined by the number of television scanning lines devoted to the detail in question. (In view of the limited resolution permitted by roentgen-television, the effect of focal-spot penumbra is, as compared with roentgenography of secondary importance.) In the second place contrast is much improved. The air gap between the patient and the image intensifier removes almost all the stray radiation, which is not directive. Removing the disturbing stray radiation background not only improves contrast but also permits more effective utilisation of the electronic amplification in the television chain the patient dose has therefore to be increased by a factor of only two instead of the theoretical factor that one

the last distributes the cytostatic agent into both the external and internal iliac arteries. It would be of advantage if thin polyethylene catheters could be introduced into the hypogastric arteries by the percutaneous technique in the same way as in selective diagnostic arteriography of the renal coeliac and mesenteric arteries. In these roentgen examinations the catheter is introduced percutaneously and the tip manoeuvred into position under roentgen television control. This technique for catheterization of the hypogastric artery has so far been used to a very small extent. *Michaelsson* 1965 described a new method by which large branches of the aorta could be catheterized easily. After some modification this technique could also be used for catheterization of the hypogastric artery. However the large diameter of the catheter (PE 240) precluded its retention in the artery for the desirable period of time. Therefore, in a few cases a thin teflon catheter was introduced via the large polyethylene catheter which was subsequently withdrawn. *Wholey and Jackman* 1966 reported on a technique by which the catheter could be manoeuvred into the hypogastric artery by a special instrument. However the catheters used had a rather large diameter of more than 2 mm. *Nilsson* 1967 published a report of 30 patients in whom one or both hypogastric arteries had been catheterized by an Ödman catheter with bent tip and with an external diameter of 2.2 mm. These techniques have been used for the diagnosis of urinary bladder tumours but tubes of such large diameter and stiffness can hardly be left in the femoral and hypogastric arteries over the period of 7-10 days which is considered as a minimum for infusion of cytostatics.

A method for percutaneous catheterization of the hypogastric artery was recently described by *Onnis et al* 1967. These authors suggested the use of Ödman catheters with a small diameter for selective catheterization of the hypogastric arteries. However even the thinnest catheters of this type are comparatively stiff and have an outer diameter of 1.8 mm.

During 1965 in a few cases we used *Michaelsson's* method to introduce thin catheters. However the technique was found to be difficult and time consuming and often failed. In 1966 a new method was introduced which was easy and reliable.

Table 1. Measured Roentgen Dose at the Image Intensifier Input A and at the Hands of the Examiner B during Hypogastric Catheterization. The Tube Voltage was 65 kV and a Geometric Magnification of 2:1 was used. Roentgen Field Size at Intensifier Input was 12x12 cm

Centre of Picture at:	A mR/h	B mR/h
Tb <sub>3</sub>	9.5	0
L <sub>1</sub>	7.5	0.2
L <sub>2</sub> S	7.0	0.6
Small pelvis	6.0	3.0

tip of the catheter to regain its original loop shape (Fig. 2 A). This can be accomplished by pushing and pulling the catheter until its tip is hooked into one of the branches of the aorta (Fig. 2 B). On pushing the catheter further it regains its original loop form, with the tip directed downwards (Fig. 2 C). In some cases this takes place spontaneously without manipulation, when the guide is withdrawn. The tip of the catheter is then withdrawn downwards to the level of the aortic bifurcation, care being taken that the tip is not introduced into some other branch of the aorta. The tip must be directed laterally (Fig. 2 D) when passing the bifurcation. The loop of the catheter is then easily withdrawn into the upper segment of the common iliac artery. Injection of contrast medium then gives information on the origin of the hypogastric artery (Fig. 2 E). The tip is then brought to this level and manoeuvred into the hypogastric artery by axial rotation (Fig. 2 F). Further axial rotation and retraction will finally bring the catheter in proper position (Fig. 2 G).

### Results

Up to the present time 19 patients have been catheterized bilaterally and two unilaterally totalling 40 catheterizations. There have been no unsuccessful attempts. Six patients suffered from choriocarcinoma, 13 had a recurrent cervical cancer and one cancer of the vulva.

The procedure has not met with technical difficulties and the

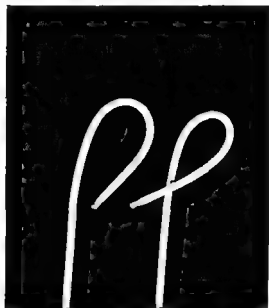


Fig. 1 The loop-formed tip of the catheter *Right* Before introduction into the artery *Left* After introduction into the aorta. During this procedure the catheter has been stretched out on the guidewire resulting in reduction of the loop form

might expect to be required by 2:1 geometrical enlargement. The catheterizations were carried out with a tube voltage of about 65 kV and a current of 0.2–0.6 mA.

Since the roentgenologist has to work closer to the roentgen field than during catheterizations of other arteries it is important that the level of stray radiation be kept as low as possible. Therefore the roentgen television assembly must be highly sensitive, the smallest possible roentgen field must be used and the roentgen tube must be placed under the examination table. The stray dose received by the examiner under these conditions are given in Table I.

The femoral artery is punctured in the groin after infiltration with local anesthetic. The catheter is then introduced into the aorta using the Seldinger technique and visualized on the monitor by filling with 76% Urographin. To introduce the catheter into the hypogastric artery the tip has to be turned downwards. Generally, the diameter of the aorta is too small to permit the





Fig. 3 39-year old woman with malignant trophoblastic tumour of the uterus. A Arteriography before treatment. The uterus is enlarged and hypervascular. Large cavities and early filling of veins are seen, indicating tumour infiltration of the uterine wall.

short periods. Continuous infusion of Methotrexate was given during 6-12 days by an electrical pump.

Fig. 3 illustrates a case with malignant trophoblastic tumour confined to the uterus and examined by arteriography before and after local infusion treatment. In this patient HCG tests soon became negative and complete regression was shown on the arteriograms.

There have been no clinical signs of thrombosis or other vas-

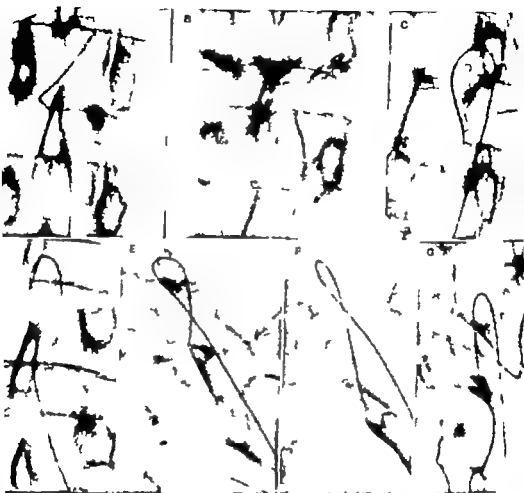


Fig 2. Series of X ray films illustrating manipulation of the catheter. A. The catheter in the aorta with the tip directed upwards. B. The tip is hooked into an aortic branch. C. After manipulation the tip is turned downwards. D. Before introduction into the common iliac artery the tip is turned laterally. E. Injection of contrast medium to localize the hypogastric artery. F. The tip is introduced into the hypogastric artery. G. Catheter in proper position after final adjustment.

period of time required for the introduction has successively decreased. At present the whole procedure with bilateral catheterization takes approximately 60 minutes or less. The catheters are maintained in proper position by fixation in the groin with surgical tape. The patient is allowed to move comparatively freely in the bed, and in some instances even leave the bed for



Fig. 3 39-year old woman with malignant trophoblastic tumour of the uterus.  
A. Arteriography before treatment. The uterus is enlarged and hypervascular.  
Large cavities and early filling of veins are seen, indicating tumour infiltration of the uterine wall.

short periods. Continuous infusion of Methotrexate was given during 6-12 days by an electrical pump.

Fig. 3 illustrates a case with malignant trophoblastic tumour confined to the uterus and examined by arteriography before and after local infusion treatment. In this patient HCG tests soon became negative and complete regression was shown on the arteriograms.

There has been no clinical signs of thrombosis or other vas-



Fig 3 B Polyethylene catheters in proper position in the hypogastric arteries. Infusion treatment with Methotrexate (50 mg/day) during 11 days combined with intramuscular injection of folic acid (6 mg every 6 hours)

cular complications. In 4 instances arteriographic examinations of the pelvic arteries were carried out within one month after the end of the infusion therapy. There was no evidence indicating damage to the arterial wall. One patient, suffering from advanced cervical carcinoma, died one week after removal of the catheters.



Fig 3. C. Arteriography 2 months after treatment indicates normal appearance of the vascular anatomy

Autopsy showed only small thromboses in the iliac arteries (Fig. 4). The localisation and extension indicate that these alterations were due to mechanical damage to the intima caused by local pressure by the catheter. Such lesions will certainly heal and are evidently without clinical importance.

#### *Discussion*

The presence of a catheter within the large arterial stems during a long period of time may give rise to complications such as thrombosis, arterio-venous fistulas, aneurysm or haematoma. The catheter should therefore have a small diameter, a smooth surface and not be too rigid. Polyethylene satisfies these criteria and also has the essential property that it may easily be formed into



Fig. 4 Autopsy specimen of the iliac arteries obtained from a patient with advanced cervical carcinoma one week after 7 days of bilateral hypogastric catheterization. The arrows indicate small thromboses within the distal part of the external iliac arteries and the left hypogastric artery. The right photograph is a magnification of the left hypogastric artery.

any desired shape after heating, contrary to most other synthetic materials.

The outer diameter and wall thickness of the polyethylene catheter determine the stiffness of the tubing. It should be firm enough to manipulate both longitudinally and axially. It should also largely regain its original loop-shaped form after withdrawal of the guide. Moreover, the inner diameter should be large enough to make possible visualization on the television-screen after injections of contrast medium.

After some trials it was found that the most suitable catheter which met the above criteria corresponded to size no. PE 100.

The Seldinger technique is the method of choice for introducing the catheter, since puncture of the femoral artery can be made with a needle of approximately the same outer diameter

as the catheter Seldinger needle no. 160 is the smallest needle that fits catheter no. PE 100. The small calibre of this needle reduces the risk of arterial complications. To be able to manipulate the catheter properly it is essential that the contrast filled tube be clearly visualized with the roentgen-television equipment. It was possible to test many different roentgen television assemblies. Most of these did not permit adequate visualization of the catheter and were therefore unacceptable.

Before inserting the infusion catheters it might be of value to study the vascular anatomy of the pelvis by a routine pelvic arteriography as related to the gynaecological pelvic examination findings. However since major vascular anomalies are rare, injection of contrast medium into the infusion catheter after insertion in the hypogastric artery in most instances gives enough information as to the appearance of the vascular anatomy and probable distribution of the cytostatic agent.

Since no complications of clinical importance occurred in 40 consecutive catheterizations it seems probable that the technique can be recommended for routine purposes. Moreover there is no need for special and complicated instruments, since ordinary Seldinger needles, guides and catheters are available in most angiographic laboratories.

For local infusion of cytostatics into the hypogastric artery there is always the problem as to how far the catheter should be introduced into the artery. If the tip of the catheter is inserted only about 1 cm there is a risk that the catheter may slip out during the infusion. If on the other hand the tip is introduced too far there is a risk that one or more main branches partially supplying the tumour area will not receive the proper concentration of the cytostatic agent. This is specially true in patients with large pelvic tumour infiltrations from recurrent cervical cancer. In the case of choriocarcinoma confined to the uterus it might be of advantage to infuse the agent more or less directly into the uterine artery. It should also be emphasized, based on many years of angiographic experience, that a catheter occluding or nearly occluding an artery may give rise to reversal of the direction of the blood flow within the artery due to alterations of the pressure gradients. As far as the uterus is concerned there is a

risk that ovarian arteries may take over the principal blood supply

If the catheter is introduced about 3 cm the tip is generally located immediately above the origin of the superior gluteal artery. In this position the catheter has no tendency to slip out and the patient can move freely in the bed and even leave the bed for short periods.

### SUMMARY

Regional chemotherapy by infusion of cytostatics into the hypogastric arteries has been used in certain cases of trophoblastic disease as well as recurrent cervical carcinoma. A method for the percutaneous introduction of thin catheters into these arteries is described. Roentgen equipment needed and technical performance of the procedure is given in detail. A total of 40 catheterizations were carried out and no complications occurred. After some training bilateral catheterization of the hypogastric arteries may be accomplished during 60 minutes or less.

### REFERENCES

- Bateman J R, Hagen J G, Stollinsky D C and Steinfeld J L. *Amer J Obstet Gynec* 96 181 1966
- Bolman R. E, Hol aepfel J H and Barnes A. C. *Amer J Obstet. Gynec* 72 1319 1956
- Cahill J J and Zeit P R. *Amer J Obstet. Gynec* 91 970 1961
- Hulka J F and Bisel H F. *Amer J Obstet. Gynec.* 91 483 1965
- Krakoff I H and Sullivan R. D. *Ann. Int Med.* 48 839 1958
- Laufe M. Personal communication
- Michaelsson C G. *Acta Radiol Diagn.* 3 581 1953
- Nilsson J. *Acta Radiol Suppl* 263 1967 (Thesis)
- Onnis A, Marsaletti G C, De Salvia D and Berilacqua L. *Amer J Obstet. Gynec* 98 966 1967
- Seldinger S I. *Acta Radiol.* 59 60 1953
- Sullivan R. D, Wood A. M, Clifford P, Duff J E, Trussell E, Mary D K. and Burchenal J R. *Cancer Chemotherap Rep.* 8 1 1960
- Trussell R. R. and Milford-Barborton G de B. *Lancet* 1 971 1961
- Wholey M H and Jackman V. *Amer J Roentgenol* 97 500 1966





## ***An important contribution to the early diagnosis of fetal distress***

**Easy correlation between contractions and heart frequency**

Researchers have established definite correlation between the well-being of the fetus and the manner in which its heart frequency is affected by uterine contractions (\*)

By simultaneously recording heart frequency and contractions, the Hewlett-Packard Model 8020A Cardiotocograph provides clear indication of this relationship.

**External sensors for safe early diagnosis**

Fetal heart sounds and labour contractions are detected by sensor which is simply placed on the mother's abdomen. No risk of infection and non-traumatic for mother and fetus. Detection of potential fetal distress can begin as soon as fetal heart sounds are audible.

After the membranes are ruptured, ECG recordings may be made with an optional ECG amplifier designed to accept a wide variety of electrodes.

**Ambient noises are rejected**

Heart frequency is computed from the two heart sounds occurring during each heart cycle. Computing errors due to ambient noise or the inaudibility of one heart sound are eliminated by unique logic circuits.

For more information on this important diagnostic instrument and its applications, write or call for your free copy of "Cardiotocography". Price HP 8020A (including strip-chart recorder and sensor) SKr 22,500.

(\*) See e.g. Hamacher K., The Diagnosis of Fetal Distress with an Electronic Fetal Heart Monitor. Proceedings of Symposium, Prague, October 1968.

**HEWLETT  PACKARD**

Sweden: Hewlett-Packard (Sverige) AB  
Sveavägen 6 S-171 20 Solna 7 tel 083 8812 50  
Högskolegatan C, 431 04 Mölndal 4  
tel 0378 77 89 00

Denmark: Hewlett-Packard A/S  
Langelinie 6 D-2650 Maribo tel 078 80 40 40

Finland: Hewlett-Packard Oy  
Göteborgin 2, Helsinki 20, tel 0738 38

Belgium: Hewlett-Packard Norge A/S  
Hedervägs 12, Oslo, tel 02 83 80





From the Institute of Medical Microbiology Department of Bacteriology (Professor O. Onckerton) University of Göteborg, and the Department of Obstetrics and Gynecology II (Professor P. Bergman) Sahlgrenska sjukhuset, Göteborg, Sweden

## STUDIES ON SERA FROM MEN WITH SPERM ANTIBODIES

BY

BO FJALLBRAND

### *Introduction*

Evidence has been presented that the sperm agglutinating factor in the blood of some men is an antibody. This factor has been connected by some investigators with sterility. In 1954 Wilson discovered that serum at high dilution from two sterile men agglutinated spermatozoa. The agglutinating activity of the sera remained after heating at 56 °C for 30 min. but was removed by absorption with semen. ABO and Rh blood group differences between sera and spermatozoa did not influence the agglutinating effect. On the basis of these studies Wilson suggested that the sperm agglutination was caused by autoantibodies. Rümke (1954) found that one of two male sperm agglutinating sera had sperm immobilizing activity which was increased in the presence of complement. Cruickshank and Stuart Smith (1959) did not find complement fixing or precipitating antibodies in two sperm agglutinating male sera studied. These investigators showed that the agglutinating activity occurred mainly in the euglobulin fraction and that absorption with AB Rh+ cells did not diminish the sperm agglutinating activity. Rümke and Hellbom (1959) after studying a larger material than the aforementioned stated that the sperm agglutinating factor was an antibody as it was heat resistant, absorbable by packed spermatozoa and detectable in the  $\gamma$ -globulin fraction after preparative paper electrophoresis. The sperm agglutinins seemed to be specific for spermatozoa and did

not agglutinate erythrocytes leucocytes or thrombocytes. Rumke and Hellenga also stated that the content of complement was normal as were the protein patterns and the rate of sedimentation of erythrocytes in all sera tested. Like Cruickshank and Stuart Smith Bandhauer (1966) found no precipitating antibodies in sperm agglutinating sera using seminal plasma. Immunofluorescence studies have been performed by Cruickshank and Stuart Smith (1959) and Sobbe *et al* (1966) who found no  $\gamma$ -globulin fixation on spermatozoa. Feltkamp *et al.* (1965) found an interrelation of agglutinating activity of sera and immunofluorescence of spermatozoa in testicular sections and sperm smears exposed to sera. With a fluorescent anti-complement serum they observed complement fixation to spermatozoa.

The author has investigated the interrelation of sperm antibodies and sterility in men (Fjällbrant 1965 1967 1968 a, b 1969 a b Fjällbrant and Obrant 1968). The aim of the present studies was to investigate the antibody character of the sperm agglutinating and immobilizing factors in sera from sterile males. Since sperm antibodies in men are looked upon as autoantibodies and since several autoimmune diseases are associated with hyper or dysgammaglobulinemia, the intent was also to investigate the level of immunoglobulins in sera with sperm antibodies.

### *Material*

#### *Blood sera*

Men with sperm agglutinins in their blood were selected from a large clinical material of male partners of female patients living in sterile or fertile marriages (Fjällbrant 1968 a). Sera from these men—all of which contained sperm antibodies—will be referred to as *patient sera*.

Blood samples were also taken from some men in fertile marriages. Sera from these men without detectable sperm antibodies will be referred to as *control sera*.

After centrifugation of the blood and decantation of the serum both the patient sera and the control sera were stored at  $-30^{\circ}\text{C}$  until analyzed.

Sera from a group of adult blood donors were used as controls

at the quantitation of immunoglobulins. No investigation with regard to the presence of sperm agglutinins was performed. These sera which were stored at +4 °C after decantation, will be referred to as *blood donor sera*.

### *Spermatozoa*

Semen samples from two donors were used for sperm antibody determinations. When not stated otherwise semen samples from a donor who belonged to blood group B were used for the antibody determinations. The volume of the samples averaged about 5 ml, the sperm density about 140 millions/ml, the percentage of motile spermatozoa about 60 the sperm motility was very rapid. Spontaneous sperm agglutination was never encountered, and the ejaculates, in other respects, were also normal.

For some experiments regarding the influence of ABO blood factors, semen samples from a donor belonging to blood group A were used. These samples were of similar quality as those from the B group donor with regard to volume sperm density per centage of motile spermatozoa and motility degree.

For the absorption studies a pool of ejaculates from many men was collected for 4 weeks and kept at +4 °C. The pool was centrifuged, the seminal plasma decanted and the spermatozoa suspended in phosphate-buffered, isotonic sodium chloride 0.1 M, pH 7.4 and recentrifuged. The supernatant was decanted, the spermatozoa were centrifuged once more and the remaining small supernatant was sucked off from the packed spermatozoa which were used for absorption.

### *Complements*

As source of complement was used pooled fresh guinea pig serum with controlled complement activity (100 % hemolysis at dilution 1 : 8 in a conventional hemolytic system). Inactivation of complement was performed by incubation at 56 °C for 30 min.

### *Methods*

Determination of sperm agglutinin titre was done with Kibrick's macroscopic direct sperm agglutination test (Kibrick *et al.* 1952)

slightly modified as described in an earlier paper (Fjällbrant 1968 a) To evaluate the precision of the method as performed by the author the following study was made

Serum from two patients one with low and the other with high sperm antibody level was dispensed in small bottles about 1 ml in each and stored at  $-30^{\circ}\text{C}$ . At intervals of 3–7 days one aliquot of each serum was thawed and inactivated. Of each aliquot 3 dilution series were prepared and the sperm agglutinin titre was determined. The procedure was repeated 5 times each time with another semen sample from the same donor. Properties of the semen samples and the results of the sperm agglutinin titre determinations are given in Table I

The range was one dilution step at low titre and two dilution steps at high titre. The standard deviation was one-half a dilution step at both low and high titres. A variation of sperm density between 94 and 196 millions/ml of the semen samples used did not seem to influence the results.

*Determination of sperm immobilizing activity* was performed as described in an earlier paper (Fjällbrant 1968 b) The employed measure of immobilizing activity of a serum or serum fraction was the time required to reduce the percentage of motile spermatozoa from about 70 to 10 in the presence of complement.

*Determination of immunoglobulin concentration* was done with the single radial immunodiffusion method (Mancini *et al.* 1965) Anti IgG anti IgA and anti IgM sera from goat (Hyland Laboratories Los Angeles Calif.) were used.

*Gel filtration* was performed at  $+4^{\circ}\text{C}$  on Sephadex G-200 using a 0.05 M phosphate buffer pH 7.0 with an addition of 0.5 M sodium chloride. The columns used were  $85 \times 2.5$  cm and the flow rate about 8 ml per hour. Aliquots of 5 ml serum mixed with 3% sucrose were added to the column. The protein content of the effluent was determined continuously at 254 m $\mu$  with an LKB Uvicord I. The effluent was collected in 5 ml portions and pooled as indicated in fig. 1 the three protein peaks comprising fractions I, II and III and the portions between fractions I and II making a fraction called m. The fractions were concentrated about 20-fold by ultrafiltration using collodium tubes (Sartorius Collodiumfilter) and dialyzed against phosphate-buffered isotonic

Table I. Sperm Agglutinin Titre of Aliquots of Two Patient Sera Determined with Five Semen Samples from the Same Donor

Semen				Sperm Agglutinin Titre			
Sample No.	Volume (ml)	Density (g/ml)	Motile Spermatozoa (%)	Motility	Patient 26	Patient 37	
1	6.0	196	62	very rapid	1 8, 1 8 1 16	1 2048, 1	2048, 1 4096
2	5.6	174	56	very rapid	1 8, 1 8 1 16	1 2048, 1	2048, 1 2048
3	6.1	128	57	very rapid	1 8 1 8 1 8	1 2048 1	2048 1 1024
4	6.0	94	68	very rapid	1 16 1 16, 1 16	1 1024 1	2048 1 2048
5	5.9	95	62	very rapid	1 16 1 8, 1 8	1 4096 1	2048, 1 2048

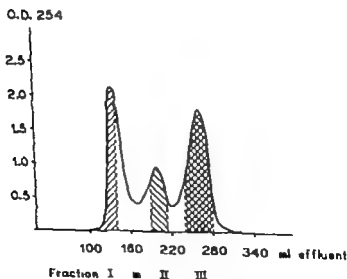


Fig 1. Gel filtration of serum on Sephadex G-200. Diagrammatic representation of fractions.

sodium chloride pH 7.4. Finally penicillin and streptomycin sulfate were added to a concentration of 3 mg/ml each.

*Reduction with mercaptoethanol* was performed by adding 0.25 ml 1 M  $\beta$  mercaptoethanol to 1 ml serum. The mixture was kept at room temperature for 4 hours and then alkylation was effected by adding 1.25 ml moniodoacetamide solution (3.7 mg per ml of isotonic sodium chloride). After 2 more hours at room temperature dialysis was performed for 3 days at +4°C against phosphate buffered isotonic sodium chloride pH 7.4. The mixture was concentrated by ultrafiltration to the original volume of 1 ml in collodium tubes.

### *Experimental*

#### *Absorption of patient sera with spermatozoa*

Aliquots of 0.5 ml of two inactivated patient sera were mixed with equal amounts of packed seminal spermatozoa from the ejaculate pool incubated at +37°C for 1 hour and at +4°C overnight. The mixtures were centrifuged, the sera decanted, and the sperm agglutinating and immobilizing activity of the absorbed sera determined. Serum samples from the same men to which no spermatozoa had been added were treated in the same way.

The unabsorbed serum samples had sperm agglutinating titres of 1:256 and 1:64 respectively. Neither of the sera had any agglutinating activity after absorption. The sperm immobilizing activity of the unabsorbed samples was 2 1/2 hours and 4 hours 20 min respectively. The immobilizing activity of the absorbed aliquots was 6 3/4 hours and 9 1/2 hours respectively.

#### *Gel filtration of patient sera*

Fractionation on Sephadex G-200 was performed on 6 patient sera and 4 control sera. The obtained fractions were investigated with regard to sperm agglutinins and sperm immobilizing antibodies. The sperm agglutinin investigation was performed at the dilutions 1:2, 1:16, 1:128 and 1:1024.

The results of the sperm agglutinin titrations are given in table II. All fractions of the control sera showed no activity and so did the first fraction from each of half of the patient sera while the others showed a weak agglutinating activity. The m fraction from



Table II. Sperm Agglutinating Activity of Fractions of Gel-Filtered Patient and Control Sera. Only Fraction Dilutions 1:2, 1:16, 1:128 and 1:1024 Were Tested

Serum No.	Sperm Agglutinating Activity				
	Before Gel Filtration	Fraction I	Fraction III	Fraction II	Fraction III
3	1:128	0	1:2	1:128	0
18	1:256	0	1:16	1:1024	0
30	1:64	1:2	1:2	1:1024	0
31	1:128	1:16	1:16	1:128	0
40	1:128	1:2	1:16	1:128	0
41	1:256	0	1:2	1:128	0
4 controls	0	0	0	0	0

all the patient sera showed low titres (1:2 or 1:16). Fraction II from each of the patient sera had sperm agglutinins at high titres (1:128 or 1:1024). All of the third fractions showed no activity. Thus sperm agglutinin activity of the patient sera was found mainly in the second peak corresponding to 7S globulins.

The results as regards sperm immobilizing activity are shown in table III. It is noted that the different fractions of the control sera differed considerably with regard to sperm immobilizing activity, the highest activity appearing in fraction I and the lowest in fraction III. The activity of fraction I of most of the patient sera was less than that of the control sera. In fraction III the activity of most of the patient sera was somewhat higher than that of the control sera. The greatest difference between the patient and the control sera was found in fraction II where the immobilizing activity of the patient sera was very much higher than that of the control sera. In fraction III the activity of the patient and control sera was rather equal. Thus sperm immobilizing activity of the patient sera was found mainly in the second peak corresponding to 7S globulins.

#### *Heat inactivation and mercaptoethanol reduction of patient sera*

Of each of 5 patient sera and 4 control sera one aliquot was inactivated at 56°C for 30 min. another aliquot was reduced with

sodium chloride pH 7.4. Finally penicillin and streptomycin sulfate were added to a concentration of 3 mg/ml each.

*Reduction with mercaptoethanol* was performed by adding 0.25 ml 1 M  $\beta$ -mercaptoethanol to 1 ml serum. The mixture was kept at room temperature for 4 hours and then alkylation was effected by adding 1.25 ml monolodoacetamide solution (37 mg per ml of isotonic sodium chloride). After 2 more hours at room temperature dialysis was performed for 3 days at +4°C against phosphate buffered isotonic sodium chloride pH 7.4. The mixture was concentrated by ultrafiltration to the original volume of 1 ml in collodion tubes.

### Experimental

#### *Absorption of patient sera with spermatozoa*

Aliquots of 0.5 ml of two inactivated patient sera were mixed with equal amounts of packed seminal spermatozoa from the ejaculate pool incubated at +37°C for 1 hour and at +4°C overnight. The mixtures were centrifuged, the sera decanted, and the sperm agglutinating and immobilizing activity of the absorbed sera determined. Serum samples from the same men to which no spermatozoa had been added were treated in the same way.

The unabsorbed serum samples had sperm agglutinating titres of 1/256 and 1/64 respectively. Neither of the sera had any agglutinating activity after absorption. The sperm immobilizing activity of the unabsorbed samples was 2 1/2 hours and 4 hours 20 min respectively. The immobilizing activity of the absorbed aliquots was 6 3/4 hours and 9 1/2 hours respectively.

#### *Gel filtration of patient sera*

Fractionation on Sephadex G-200 was performed on 8 patient sera and 4 control sera. The obtained fractions were investigated with regard to sperm agglutinins and sperm immobilizing antibodies. The sperm agglutinin investigation was performed at the dilutions 1/2, 1/16, 1/128 and 1/1024.

The results of the sperm agglutinin titrations are given in table II. All fractions of the control sera showed no activity and so did the first fraction from each of half of the patient sera while the others showed a weak agglutinating activity. The m fraction from

Table IV *Sperm Immobilizing Activity of Patient and Control Sera after Treatment*

Serum No.	Sperm Immobilizing Activity (in hours)			
	Untreated	Heated 56° C 30 min.	Reduced with Mercaptoethanol	Dialyzed with Saline
18	5 1/2	3 1/2	8 1/2	10
30	8 1/2	7 1/2	12	16
31	4	4	12	12
40	4 1/2	3 3/4	11	11
41	8 1/2	6 1/2	17	17
4 controls				
mean	47	44	40	44
range	45-48	43-45	39-40	43-44

patient sera were determined after the addition of fresh guinea pig serum and inactivated guinea pig serum. Two dilution series were made up of each serum. In one series 0.1 ml of the 0.3 ml of serum dilution usually used with the method of Kibrick was replaced by fresh guinea pig serum, in the other series by 0.1 ml inactivated guinea pig serum.

The sperm agglutinin titres of the sera were after addition of fresh guinea pig serum 1:128, 1:4096 and 1:16 respectively. With the addition of inactivated guinea pig serum the titres were 1:256, 1:2048 and 1:32, respectively.

2. To investigate the influence of complement on the activity of sperm immobilizing antibodies the immobilizing activities of 6 inactivated patient sera and one inactivated control serum were determined. Two mixtures were made up of each serum, one containing fresh guinea pig serum and the other inactivated guinea pig serum.

The results are given in Table V. The immobilizing activity of all patient sera was considerably lower in the absence of complement.

#### *Influence of the ABO blood group system on sperm agglutinating and immobilizing activity*

1. Serum samples from 17 men selected at random from the sterility clientele and belonging to various ABO blood groups were

Table III. *Sperm Immobilizing Activity of Fractions of Gel-Filtered Patient and Control Sera*

Serum No.	Sperm Immobilizing Activity (in hours)				
	Before Gel Filtration	Fraction I	Fraction m	Fraction II	Fraction III
3	4 ½	27	14	3 ½	60
18	5 ¼	14	17	6 ½	54
30	8 ½	20	22	■	58
31	4	23	11	4 ¼	54
40	4 ¼	22	12	3 ¼	54
41	8 ½	26	13	7	55
4 controls					
mean	47	13	19	26	51
range	45-48	11-14	16-24	23-28	48-54

*mercaptoethanol* and a third aliquot was *dialy cd* against phosphate-buffered isotonic sodium chloride for 3 days in the same way as the *mercaptoethanol* reduced aliquots. The sperm agglutinin titre of the untreated inactivated and reduced aliquots was determined as was the sperm immobilizing activity of all the aliquots.

The sperm agglutinin titres of the inactivated and reduced aliquots of patient sera were the same or varied within one dilution step. The untreated inactivated and reduced control sera showed no agglutinating activity.

As regards sperm immobilizing activity (see Table IV) this was generally somewhat higher in the inactivated than in the untreated aliquots of patient sera. The immobilizing activity of the *mercaptoethanol* reduced aliquots of patient sera was lower than that of the inactivated and untreated aliquots but of the same magnitude as the activity of those aliquots which were only dialyzed. The various aliquots of control sera had about the same sperm immobilizing activity with a tendency to higher activity in the *mercaptoethanol* reduced aliquots.

#### *Influence of complement*

1 To investigate the influence of complement on the activity of sperm agglutinins the sperm agglutinin titres of 3 inactivated

Table IV *Sperm Immobilizing Activity of Patient and Control Sera after Treatment*

Serum No.	Sperm Immobilizing Activity (in hours)			
	Untreated	Heated 56° C 30 min.	Reduced with Mercaptoethanol	Dialyzed with Saline
III	5 /	3 ½	8 ½	10
30	8 ½	7 ½	12	16
31	4	4	12	12
40	4 /	3 ¾	11	11
41	8 ½	6 ½	17	17
4 controls				
mean	47	44	40	44
range	45-48	43-45	39-40	43-44

patient sera were determined after the addition of fresh guinea pig serum and inactivated guinea pig serum. Two dilution series were made up of each serum. In one series 0.1 ml of the 0.3 ml of serum dilution usually used with the method of Kibrick was replaced by fresh guinea pig serum, in the other series by 0.1 ml inactivated guinea pig serum.

The sperm agglutinin titres of the sera were after addition of fresh guinea pig serum 1:128, 1:4096 and 1:16 respectively. With the addition of inactivated guinea pig serum the titres were 1:256, 1:2048 and 1:32 respectively.

2. To investigate the influence of complement on the activity of sperm immobilizing antibodies, the immobilizing activities of 6 inactivated patient sera and one inactivated control serum were determined. Two mixtures were made up of each serum, one containing fresh guinea pig serum and the other inactivated guinea pig serum.

The results are given in Table V. The immobilizing activity of all patient sera was considerably lower in the absence of complement.

#### *Influence of the ABO blood group system on sperm agglutinating and immobilizing activity*

1. Serum samples from 17 men selected at random from the sterility clientele and belonging to various ABO blood groups were

Table III Sperm Immobilizing Activity of Fractions of Gel-Filtered Patient and Control Sera

Sera No.	Sperm Immobilizing Activity (in hours)				
	Before Gel Filtration	Fraction I	Fraction II	Fraction III	Fraction IV
3	4 1/2	27	14	3 1/2	60
18	5 1/4	14	17	6 1/2	54
30	8 1/2	20	22	6	58
31	4	23	11	4 1/4	54
40	4 1/4	22	12	3 1/4	54
41	8 1/2	26	13	7	55
4 controls					
mean	47	13	19	26	51
range	45-48	11-14	16-24	23-28	48-54

mercaptoethanol and a third aliquot was dialyzed against phosphate-buffered isotonic sodium chloride for 3 days in the same way as the mercaptoethanol reduced aliquots. The sperm agglutinin titre of the untreated inactivated and reduced aliquots was determined as was the sperm immobilizing activity of all the aliquots.

The sperm agglutinin titres of the inactivated and reduced aliquots of patient sera were the same or varied within one dilution step. The untreated, inactivated and reduced control sera showed no agglutinating activity.

As regards sperm immobilizing activity (see Table IV) this was generally somewhat higher in the inactivated than in the untreated aliquots of patient sera. The immobilizing activity of the mercaptoethanol-reduced aliquots of patient sera was lower than that of the inactivated and untreated aliquots but of the same magnitude as the activity of those aliquots which were only dialyzed. The various aliquots of control sera had about the same sperm immobilizing activity with a tendency to higher activity in the mercaptoethanol reduced aliquots.

#### *Influence of complement*

1 To investigate the influence of complement on the activity of sperm agglutinin the sperm agglutinin titres of 3 inactivated

Table IV Sperm Immobilizing Activity of Patient and Control Sera after Treatment

Serum No.	Sperm Immobilizing Activity (in hours)			
	Untreated	Heated 56° C 30 min.	Reduced with Mercaptoethanol	Dialyzed with Saline
18	5 1/2	3 1/2	8 1/2	10
30	8 1/2	7 1/2	12	16
31	4	4	12	12
40	4 1/2	3 1/2	11	11
41	8 1/2	6 1/2	17	17
4 controls				
mean	47	44	40	44
range	45-48	43-45	39-40	43-44

patient sera were determined after the addition of fresh guinea pig serum and inactivated guinea pig serum. Two dilution series were made up of each serum. In one series 0.1 ml of the 0.3 ml of serum dilution usually used with the method of Kibrick was replaced by fresh guinea pig serum, in the other series by 0.1 ml inactivated guinea pig serum.

The sperm agglutinin titres of the sera were after addition of fresh guinea pig serum 1:128, 1:4096 and 1:16 respectively. With the addition of inactivated guinea pig serum the titres were 1:256, 1:2048 and 1:32 respectively.

2. To investigate the influence of complement on the activity of sperm immobilizing antibodies the immobilizing activities of 6 inactivated patient sera and one inactivated control serum were determined. Two mixtures were made up of each serum, one containing fresh guinea pig serum and the other inactivated guinea pig serum.

The results are given in Table V. The immobilizing activity of all patient sera was considerably lower in the absence of complement.

#### *Influence of the ABO blood group system on sperm agglutinating and immobilizing activity*

1. Serum samples from 17 men selected at random from the sterility clientele and belonging to various ABO blood groups were

Table V *Sperm Immobilizing Activity after Addition of Fresh Guinea Pig Serum as Source of Complement and Addition of Inactivated Guinea Pig Serum*

Serum No	Sperm Immobilizing Activity (in hours)	
	With Complement	Without Complement
37	2 ¼	8 ¼
45	4 ½	23
41	5 ¼	17
35	6 ¼	52
43	22	52
44	36	47
Control	60	60

investigated for sperm agglutinins (at dilution 1 : 4) with semen both from a donor belonging to blood group A and one belonging to blood group B. One serum was sperm agglutinin positive with both semen samples and 16 sera were negative.

2. With semen samples from both the A and B donors the sperm agglutinin titres of 12 patient sera from men in the sterility clientele who belonged to group A, B, O or AB were determined. The sperm agglutinin titres obtained with the unlike semen samples were the same or showed no significant difference.

3. Aliquots of 5 patient sera, 3 from men belonging to blood group A and 2 belonging to blood group O were absorbed with blood group specific substance II until no agglutination of B erythrocytes occurred. The sperm agglutinin titre of one absorbed and one unabsorbed aliquot of each serum was determined with semen from a donor belonging to blood group B who was a secretor of B substance in his semen.

The sperm agglutinin titres of the unabsorbed A sera were 1 : 512, 1 : 256 and 1 : 256 respectively; the titres of the corresponding absorbed sera were 1 : 512, 1 : 256 and 1 : 64. The titres of the unabsorbed O sera were 1 : 8 and 1 : 16; the titres of the absorbed sera were the same.

The sperm immobilizing activity of the unabsorbed and B-substance-absorbed aliquots of two of the aforementioned A sera was



determined with semen from the same B group donor. The sperm immobilizing activity of the absorbed and unabsorbed aliquots of each serum was the same 2 h and 5 1/2 h, respectively.

4. An aliquot of patient serum from a man belonging to blood group B was absorbed with A substance and A erythrocytes. The sperm agglutinin titre of one absorbed and one unabsorbed aliquot of the serum was determined with semen both from a donor belonging to group A and one belonging to group B.

The sperm agglutinin titre of the unabsorbed serum was 1:1024 with both the A and the B semen; the titre of the absorbed serum was 1:1024 with A semen and 1:2048 with B semen.

The immobilizing activity of the absorbed and unabsorbed B serum aliquots was determined with semen from the A donor. The sperm immobilizing activity was the same with both aliquots (6 h).

#### *Immunoglobulin levels of patient and blood donor sera*

The concentration of IgG, IgA and IgM was determined in 40 patient sera and in 40 blood donor sera.

The results, given as mean, standard deviation and range of the concentration of each immunoglobulin within the patient and blood donor groups are indicated in Table VI. The men with sperm antibodies had a higher mean level of IgG and IgM but a lower mean level of IgA than the blood donors. Statistical analysis showed that the difference between the groups with regard to the means for IgG was significant ( $t=3.23$ ,  $p<0.01$ ) and so was the difference with regard to the means for IgM ( $t=2.65$ ,  $p<0.01$ ).

The difference with regard to the means for IgA was not significant ( $t=-1.32$ ,  $p>0.05$ ).

#### *Discussion*

Absorption of immune sera by means of antigens is a classical method for depleting sera immunospecifically of antibody activity.

The tests were performed at the Municipal Bacteriological Laboratory & Carlsberg Section of Immunology in collaboration with Dr. Lars-Alke Nil-

Table V *Sperm Immobilizing Activity after Addition of Fresh Guinea Pig Serum as Source of Complement and Addition of Inactivated Guinea Pig Serum*

Serum No.	Sperm Immobilizing Activity (in hours)	
	With Complement	Without Complement
37	2 ¼	8 ¼
45	4 ½	23
41	5 ¼	17
35	6 ¼	52
43	22	52
44	36	47
Control	60	60

investigated for sperm agglutinins (at dilution 1 : 4) with semen both from a donor belonging to blood group A and one belonging to blood group B. One serum was sperm agglutinin positive with both semen samples and 16 sera were negative.

2. With semen samples from both the A and B donors the sperm agglutinin titres of 12 patient sera from men in the sterility clientele who belonged to group A, B, O or AB were determined. The sperm agglutinin titres obtained with the unlike semen samples were the same or showed no significant difference.

3. Aliquots of 5 patient sera, 3 from men belonging to blood group A and 2 belonging to blood group O were absorbed with blood group specific substance B until no agglutination of B erythrocytes occurred. The sperm agglutinin titre of one absorbed and one unabsorbed aliquot of each serum was determined with semen from a donor belonging to blood group B who was a secretor of B substance in his semen.

The sperm agglutinin titres of the unabsorbed A sera were 1 : 512, 1 : 256 and 1 : 256 respectively; the titres of the corresponding absorbed sera were 1 : 512, 1 : 256 and 1 : 64. The titres of the unabsorbed O sera were 1 : 8 and 1 : 16; the titres of the absorbed sera were the same.

The sperm immobilizing activity of the unabsorbed and B-substance-absorbed aliquots of two of the aforementioned A sera was

determined with semen from the same B group donor. The sperm immobilizing activity of the absorbed and unabsorbed aliquots of each serum was the same 2 h and 5 1/2 h, respectively.

4. An aliquot of patient serum from a man belonging to blood group B was absorbed with A substance and A erythrocytes. The sperm agglutinin titre of one absorbed and one unabsorbed aliquot of the serum was determined with semen both from a donor belonging to group A and one belonging to group B.

The sperm agglutinin titre of the unabsorbed serum was 1:1024 with both the A and the B semen; the titre of the absorbed serum was 1:1024 with A semen and 1:2048 with B semen.

The immobilizing activity of the absorbed and unabsorbed B serum aliquots was determined with semen from the A donor. The sperm immobilizing activity was the same with both aliquots (6 h).

#### *Immunoglobulin levels of patient and blood donor sera*

The concentration of IgG, IgA and IgM was determined in 40 patient sera and in 40 blood donor sera.

The results, given as mean, standard deviation and range of the concentration of each immunoglobulin within the patient and blood donor groups are indicated in Table VI. The men with sperm antibodies had a higher mean level of IgG and IgM, but a lower mean level of IgA than the blood donors. Statistical analysis showed that the difference between the groups with regard to the means for IgG was significant ( $t=3.23$ ,  $p<0.01$ ) and so was the difference with regard to the means for IgM ( $t=2.65$ ,  $p<0.01$ ). The difference with regard to the means for IgA was not significant ( $t=-1.32$ ,  $p>0.05$ ).

#### *Discussion*

Absorption of immune sera by means of antigens is a classical method for depleting sera immunospecifically of antibody activity.

The tests were performed at the Municipal Bacteriological Laboratory of Göteborg, Section of Immunology in collaboration with Dr. Lars-Ake Nilsson.

Table VI. Concentrations (mg/100 ml) of Immunoglobulins in Male Patients with Sperm Antibodies and 40 Blood Donors

	Patients			Blood Donors	
	IgG	IgA	IgM	IgG	IgA
Mean	1830	192	94	1533	222
Standard deviation	495	67	43	302	127
Range	940-3160	27-360	43-250	1050-2450	59-

In the author's absorption experiments with sperm and immobilizing sera a complete exhaustion of agglutivity could be obtained by treating the sera with sperm. The immobilizing activity however was only reduced. The same procedure and could not be completely removed in relation with spermatozoa.

Since spermatozoa are carriers of A and B blood group (Gullbring, 1957) it could be expected that blood group antigens might influence the sperm agglutinating activity. Absorption of patient sera with blood group substances however decrease the sperm agglutinating or immobilizing activity. This finding indicates that the serological activity of patient sera as revealed by the agglutination and immobilization tests is not caused by antibodies against blood group substances. The agglutinating activity of sera was also tested with spermatozoa from semen donors of different blood groups. It was found that this use of semen from donors with different blood groups did not influence the results of the agglutination test. This observation conforms with the aforementioned lack of influence of the ABO blood group agglutinins. Statistical analysis of the distribution of ABO blood groups in large sperm agglutinin positive and negative groups have also pointed to a lack of influence of the ABO blood group system on the sperm agglutination (Fjällbrant 1968 a and 1969 a).

After gel filtration most of the agglutinating activity was found in the second peak. As this peak is known to contain the 7S antibodies the finding indicates that the sperm agglutinating anti-

bodies are probably of the 7S type. A weak sperm agglutinating activity was found in the first peak of some sera, and therefore there may be some agglutinating antibodies with larger molecular size. The results of mercaptoethanol reduction suggest that these antibodies of larger size represent only a minor part of the agglutinating activity of serum. Sulphydryl compounds such as  $\beta$ -mercaptoethanol dissociate 19S antibodies into smaller units. The unchanged agglutinating activity of sera after reduction with mercaptoethanol indicates that the agglutinating antibodies are not 19S antibodies and thus aids in characterizing the sperm agglutinating antibodies as 7S antibodies.

The second peak obtained at gel filtration of patient sera showed a sperm immobilizing activity which was markedly higher than that of the other peaks. Control sera also showed immobilizing activity in the second peak after gel filtration but the activity of the second peak of patient sera was distinctly higher than that of the control sera. These results indicate that the sperm immobilizing antibodies of patient sera are mainly of the 7S type. It is noted, however that in the control sera the sperm immobilizing activity of the three peaks differed between themselves, the highest activity appearing in the first peak and the lowest in the third. This finding points to the existence of other sperm immobilizing factors, an observation which deserves further investigation. The immobilizing activity was decreased by mercaptoethanol reduction, a procedure that necessitated extensive dialysis before testing of the activity but this decrease cannot explicitly be assumed to be caused by the reduction process, because the decrease of immobilizing activity was of the same magnitude when the sera were only dialyzed.

In the investigations of the incidence of sperm antibodies in groups of men with different fertility performed by the author the sera were inactivated at 56°C for 30 min. before their examination for sperm agglutinating and immobilizing activity. The studies reported on in this paper show that such an inactivation can be performed without decrease of the antibody activity in comparison to that of the untreated serum.

The presence of complement did not influence the activity of the agglutinating antibodies but considerably enhanced that of

Table VI. Concentrations (mg/100 ml) of Immunoglobulins in the Sera of 40 Male Patients with Sperm Antibodies and 40 Blood Donors

	Patients			Blood Donors		
	IgG	IgA	IgM	IgG	IgA	IgM
Mean	1830	192	94	1533	222	72
Standard deviation	495	67	43	302	127	30
Range	940-3160	27-360	43-250	1050-2450	59-545	32-168

In the author's absorption experiments with sperm agglutinating and immobilizing sera a complete exhaustion of agglutinating activity could be obtained by treating the sera with spermatozoa. The immobilizing activity however was only reduced by the same procedure and could not be completely removed by absorption with spermatozoa.

Since spermatozoa are carriers of A and B blood group factors (Gullbring, 1957) it could be expected that blood group agglutinins might influence the sperm agglutinating activity of serum. Absorption of patient sera with blood group substances did not, however decrease the sperm agglutinating or immobilizing activity. This finding indicates that the serological activity of the patient sera as revealed by the agglutination and immobilization tests is not caused by antibodies against blood group substances. The agglutinating activity of sera was also tested with spermatozoa from semen donors of different blood groups. It was noted that this use of semen from donors with different blood groups did not influence the results of the agglutination test. This observation conforms with the aforementioned lack of influence of the ABO blood group agglutinins. Statistical analysis of the distribution of ABO blood groups in large sperm agglutinin positive and negative groups have also pointed to a lack of influence of the ABO blood group system on the sperm agglutination test (Fjällbrant 1968 a and 1969 a).

After gel filtration most of the agglutinating activity was found in the second peak. As this peak is known to contain the 7S antibodies the finding indicates that the sperm agglutinating anti

complement. The levels of IgG, IgA and IgM were determined in 40 sera with sperm antibodies and 40 blood donor sera.

Absorption with seminal spermatozoa completely exhausted the sperm agglutinating activity but removed only partially the immobilizing activity. Absorption with blood group substances did not influence the antibody activity of the sera. The sperm agglutinating antibodies and probably also the immobilizing antibodies were mainly of the 7S type. Incubation at 36°C for 30 min. did not decrease the sperm agglutinating or immobilizing activity. Presence of complement enhanced the immunomobilization of spermatozoa but did not influence the activity of the agglutinins. The levels of IgG and IgM were significantly higher in sera from men with sperm antibodies than in control sera ( $p < 0.01$ ).

#### REFERENCES

- Bendhauer K., Immunreaktionen bei Fertilitätsstörungen des Mannes. *Urol. int. (Basel)* 21: 247, 1966.
- Cricklake B. and Smart Smith D. A., Orchitis associated with sperm-agglutinating antibodies. *Lancet* i: 708, 1959.
- Fikamp T. E. W., Kravoff K., Ledger, N. C. J. J. and Rainier P. Auto-spermagglutination: immunofluorescent studies. *Ann. N.Y. Acad. Sci.* 124: 702, 1965.
- Füllbrunn B. Immunagglutination of sperm in cases of sterility. *Acta obstet. gynec. scand.* 44: 474, 1965.
- Fertility is also sensitized to sperm. *J. Reprod. Fertil.* 14: 143, 1967.
- Sperm agglutination in sterile and fertile men. *Acta obstet. gynec. scand.* 47: 89, 1968.
- Lower relation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility in men. *Acta obstet. gynec. scand.* 47: 102, 1968b.
- Sperm agglutination in male blood donors. *Acta obstet. gynec. scand.* 48: 64, 1969.
- Cervical mucus permeation by human spermatozoa treated with anti-spermatozoal antibodies from rabbit and man. *Acta obstet. gynec. scand.* 48: 71, 1969b.
- Füllbrunn B. and Obrast O. Clinical and seminal findings in men with sperm antibodies. *Acta obstet. gynec. scand.* 47: 451, 1968.
- Gullbrugg, B. Investigation on the occurrence of blood group antigens in spermatozoa from man, and serological demonstration of the segregation of characters. *Acta med. scand.* 159: 169, 1957.
- Hiras C. F. J. and Boyer J. T. Dysgammaglobulinemia in the adult manifested as autoimmune hemolytic anemia. Serologic and immunochemical

the immobilizing antibodies which conforms with the finding of *Rumke* (1954). This immobilization of spermatozoa mediated by antibody and complement suggests that the process is a cytotoxic one and is parallel to the immobilization phenomenon at e.g. the TPI test (*Nelson and Mayer, 1949*).

Systemic lupus erythematosus is almost invariably accompanied by marked diffuse hypergammaglobulinemia and many other autoimmune diseases e.g. rheumatoid arthritis, polyarteritis nodosa, ulcerative colitis and Hashimoto's thyroiditis are often associated with moderate hypergammaglobulinemia (*Janeway et al. 1967*). Type I dysgammaglobulinemia which involves deficiency of IgA and IgG globulins and increased amounts of IgM globulin can be accompanied by autoimmune processes (*Hin and Boyer 1963*). In the present investigation the concentration of immunoglobulins was determined in 40 patient sera and 40 blood donor sera. The blood donor sera were not investigated with regard to the presence of sperm agglutinins but as the incidence of sperm agglutinin positivity was 4.8 per cent in male blood donor sera (*Fjällbrant 1968 d*) this circumstance can not be expected to diminish the value of the blood donor sera as controls to any significant degree. The immunoglobulin levels in the blood donors were within the usually indicated range of normal values with the exception of the mean of IgG concentration which was slightly higher than usually found. The mean concentrations of IgG and IgM in the sera from patients with sperm antibodies were statistically significantly higher than the means of the blood donor sera. This observation of raised immunoglobulin levels in sera with sperm antibodies suggests that formation of measurable sperm antibody levels in men is associated with a generally increased production of antibodies.

## SUMMARY

Male sera with sperm agglutinating and immobilizing activity were studied by absorption with seminal spermatozoa and with A and B blood group substances by filtration on Sephadex G-200 reduction with mercaptoethanol and incubation at 56 °C for 30 min. The activity was also determined in the presence and absence of



## CYCLICAL VARIATIONS IN THE ARBORACEOUS CRYSTAL PATTERN OF AIR-DRIED SEMINAL FLUID

BY

J. E. KIHLSSTRÖM AND D. FJELLSTRÖM

For a long time it has been looked upon as a fact that while the female sexual functions are cyclical in nature the male sexual functions are not (Bullough 1951). This conception seems to be supported by the studies of the sexual differentiation of the brain (Harris 1964). However the existence in both sexes of similar feed-back mechanisms of interacting gonadotrophic and gonadal hormones makes it plausible to expect the occurrence of cyclic phenomena, corresponding to the oestrous cycle in the male also. Such rhythmic changes were first observed in rabbits (Doggett 1956 Kihlström 1958) and have since been demonstrated to occur in cattle (Kihlström, 1962) rats (Kihlström 1963) mice (Kihlström and Järnebrant 1968) and possibly also in man (Doggett and Kellers 1962 Hornstein 1965 Månsson 1965). In all species so far studied the duration of this male cycle is approximately the same as that of the corresponding female sexual cycle.

The most important parameters shown to vary cyclically are the volume of ejaculate (Kihlström 1958 Kihlström 1963 Kihlström and Hultén 1968 Degerman and Kihlström 1961) the number of sperm cells (Doggett 1956 Doggett and Kellers 1962 Kihlström 1963 Kihlström and Hultén 1968) the sexual drive (Degerman and Kihlström 1961) the fertility (Kihlström 1962, 1963 Kihlström and Hultén 1968) the exfoliation of cells from the urethra (Kihlström and Hornstein 1964 Hornstein 1965 Kihlström and Järnebrant 1968) the spontaneous ejaculations

- characterization of an antibody of unusual specificity *New Eng. J Med.* 269 1329 1963
- Janeeway C. A. Rosen F. S. Merler E. and Alper C. A.* The gamma globulins. Little Brown and Company Boston 1967
- Kibrick S. Belding, D. L. and Merrill B.* Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. *Fertil. and Steril.* 3 430 1952
- Mancini G. Carbonara A. O. and Heremans J. F.* Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2 235, 1965
- Nelson R. A. Jr and Mayer M. M.* Immobilization of *treponema pallidum* in vitro by antibody produced in syphilitic infection. *J. exp. Med.* 89 369 1949
- Rünke P.* The presence of sperm antibodies in the serum of two patients with oligozoospermia *Vox. Sang. (Basel)* 4 135 1954
- Rünke P. and Hellings G.* Autoantibodies against spermatozoa in sterile men. *Amer. J. clin. Path.* 32 357 1959
- Wilson L.* Sperm agglutinins in human semen and blood. *Proc. Soc. exp. Biol. (N. Y.)* 85 652 1954

Received on March 12, 1968

## CYCLICAL VARIATIONS IN THE ARBORACEOUS CRYSTAL PATTERN OF AIR-DRIED SEMINAL FLUID

BY

J. E. KIHLSTRÖM AND D. FJELLSTRÖM

For a long time it has been looked upon as a fact that while the female sexual functions are cyclical in nature, the male sexual functions are not (Bullough 1951). This conception seems to be supported by the studies of the sexual differentiation of the brain (Harris 1964). However the existence in both sexes of similar feed-back mechanisms of interacting gonadotrophic and gonadal hormones makes it plausible to expect the occurrence of cyclic phenomena, corresponding to the oestrous cycle, in the male also. Such rhythmic changes were first observed in rabbits (Doggott 1956 Kihlström, 1958) and have since been demonstrated to occur in cattle (Kihlström 1962) rats (Kihlström 1965) mice (Kihlström and Järnebrant 1968) and possibly also in man (Doggott and Kellers 1962 Hornstein 1965 Månsson 1965). In all species so far studied the duration of this male cycle is approximately the same as that of the corresponding female sexual cycle.

The most important parameters shown to vary cyclically are the volume of ejaculate (Kihlström 1958 Kihlström 1963 Kihlström and Hultnäs 1968 Degerman and Kihlström 1961) the number of sperm cells (Doggott 1956 Doggott and Kellers 1962 Kihlström 1963 Kihlström and Hultnäs 1968) the sexual drive (Degerman and Kihlström 1961) the fertility (Kihlström 1962, 1963 Kihlström and Hultnäs 1968) the exfoliation of cells from the urethra (Kihlström and Hornstein 1964 Hornstein 1965 Kihlström and Järnebrant 1968) the spontaneous ejaculations

- characterization of an antibody of unusual specificity *New Eng. J Med.* 269 1329 1963
- Janeway C. A. Rosen F. S. Merler E. and Alper C. A.* The gamma globulins. Little Brown and Company Boston 1967
- Kibrick S. Bolding, D. L. and Merrill B.* Methods for the detection of antibodies against mammalian spermatozoa. II. A gelatin agglutination test. *Fertil. and Steril* 3 430 1952
- Manchini G. Carbonara A. O. and Heremans J. F.* Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2 235 1965
- Nelson R. A. Jr. and Mayer M. M.* Immobilization of *treponema pallidum* in vitro by antibody produced in syphilitic infection. *J. exp. Med.* 89 369 1949
- Rümke P.* The presence of sperm antibodies in the serum of two patients with oligozoospermia. *Vox. Sang (Basel)* 4 135 1954
- Rümke P. and Hellings G.* Autoantibodies against spermatozoa in sterile men. *Amer J clin. Path.* 32 357 1959
- Wilson L.* Sperm agglutinins in human semen and blood *Proc. Soc. exp. Biol. (N. Y.)* 85 652, 1954

Received on March 12 1968

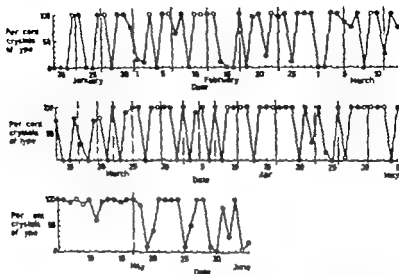


Fig 1 Daily variation during six months of the percentage of crystals of type R in the representative area of the preparations. Dotted lines mark the mid-points of the periods during which crystals of type R occur in the preparations.

temperature of the room was thermostatically regulated, and the food was always given at the same time of the day. Semen was collected once a day between 1 and 2 p.m. and delivered into a graduated test tube, used in determining the volume of the ejaculates.

The ejaculates were immediately freed from spermatozoa by centrifuging for 10 minutes at 975 g. A small drop of the seminal fluid was placed on a well cleaned object glass-slide and dried in air at a temperature of 21-23°C. The preparations obtained in this way were permanent and easy to study under the microscope.

As the decreasing thickness at the periphery of the drops may influence the forms of the crystals only a central area of each preparation, defined in the following way, was studied. By using a microprojector an image (magnification 120 $\times$ ) of the dried drop was drawn on millimeter-squared paper. A circle, as large as possible, was then inscribed in the drawing of the dried drop.

(Kihlström 1965) and the body temperature (Degerman and Kihlström 1964). Some experiments also indicate a hormonal regulation of these cyclic changes (Hornstein Kihlström and Degerman 1964 Inoué 1965 a and b Exley and Corker 1965). A review of the literature is given by Kihlström (1966).

In the females the characteristic palm leaf like crystals appearing in dried samples of cervical mucus, first observed by Papanicolaou (1933) occur abundantly around the day of ovulation, but are uncommon or even completely missing during other parts of the menstrual cycle (Rydberg, 1948). As early as 1921 similar crystals were observed in semen from cattle horse and rabbit (Yamane 1921). Nevertheless the occurrence of such crystals in semen has later been denied (Rydberg, 1948 Rodrique Villa 1958). Similar crystals also appear in the secretions from the prostate (Rodrique, Villa 1956 1958 Vartapetov and Demtjenko 1965) and the urethral glands (Belonoschikín 1964). Arboraceous crystals in semen, if they exist, could possibly be expected to be subject to cyclic changes such as those seen in the cervical mucus. Moreover the characteristics of the crystals are possibly less dependent upon extraneous factors than are the different expressions of the sexual activity for example the volume of ejaculate earlier used in studying the male sexual cycle. For these reasons we tried to confirm Yamane's observations in order to follow the daily variation of crystallization in samples of semen.

### *Material and Methods*

Semen was collected from 12 rabbits (Swedish Country Breed) and from 21 bulls (Swedish Red Breed) by means of artificial vaginas and from two men by masturbation.

One of the rabbits was used for a continuous study of the crystals in dried seminal fluid from ejaculates yielded once a day from the middle of January to the beginning of June. For this particular buck the mean duration of the male sexual cycle was known from earlier studies to be between 6 and 7 days (Degerman and Kihlström 1968). The animal was kept in a separate cage in a windowless room and subjected to an artificial cycle of daylight with a dark period extending from 8 a.m. to 8 p.m. The



Fig. 2 Crystals of type R. Magnification 1030  $\times$

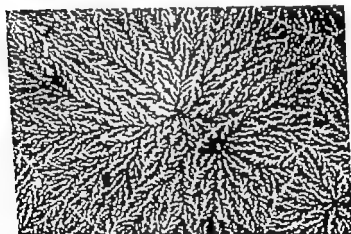


Fig. 3 Crystals of type A. Magnification 1030  $\times$

are perpendicular to the stems. The branches as well as the stems are practically straight. In a very few cases only the stems diverge from a common centre. The second pattern of crystals (type A, acute-angled crystals, Fig. 3) has branches forming acute angles

Another circle with the same centre and a radius half that of the large one was taken as the representative area. Within this area the zones containing different types of crystals were marked on the paper and calculated as percentages of the total surface with an accuracy of  $\pm 2.5$  per cent. The figures obtained in this way were arranged chronologically with a 24-hour period as a constant interval of time. A series thus obtained is diagrammatically represented in Fig. 1. The method of Hermack and McKendrick (1937) was used to investigate whether the different crystallization patterns appear at random or not. More detailed information about a possibly occurring cyclic variation was obtained by applying a serial  $\chi^2$  test, related to the method of serial correlation, as follows. Within the chronological series mentioned above the data were classified into two categories, for example one representing preparations with more than 50 per cent crystals of type R (see below) and the other representing preparations with less than this figure. The series was then displaced along its own copy 1, 2, 3 etc. time units (one time unit = 24 hours). After every displacement the number of data in the undisplaced series coinciding with data of the same category in the displaced series were noted. The number of coinciding pairs thus obtained was then compared by means of the  $\chi^2$  test with the corresponding number calculated for a random distribution of the data of the two classes. A cyclic variation, if existing, will manifest itself as a higher number of coinciding pairs than expected. This will happen when the length of the displacement is close to that of the cycle or to a multiple of that length.

### Results

After eliminating spermatozoa and other particles from the ejaculates palm leaf like crystals are seen in about 50 per cent of the samples from bulls and men and in the overwhelming majority of the samples from rabbits. For various reasons a more detailed study was restricted to rabbit semen.

In air-dried preparations of seminal fluid from rabbits two distinctly different types of ramified crystals were observed. In the first type (type R, rightangled crystals Fig. 2) the branches



with the stems. The branches as well as the stems are gently undulating and a number of stems diverge from a common point in the centre of each crystal. When the crystals of this type occur abundantly and close together they form a polygonal pattern (Fig. 4). In addition crystals having a form transitional between those of type R and type A (Fig. 5) may be seen.

As seen from the photomicrographs in Fig. 6 and the diagram in Fig. 1 the form of the crystals in seminal plasma from the rabbit studied serially varied from day to day in a more or less regular way. Most of the preparations were dominated by crystals of type R, sometimes intermingled with the transitional form of crystals. The occurrence of the crystals of type A was more restricted, and the polygonal pattern appeared sparsely and rarely dominated the preparations. As seen from Table I the distribution of these different patterns of crystallization is far from random, which strongly indicates the existence of a cyclic variation.

The results arrived at when applying the serial  $\chi^2$ -test described above are given in Table II. As seen from the table the occurrence of preparations with crystals of type R as the predominant type shows a statistically significant rhythmicity with a length of seven days. The third multiple of this length also gives a statistically significant value. Preparations containing exclusively crystals of type R give statistically significant values, when the length of the displacement is a multiple of seven. We are thus compelled to conclude that the crystallization pattern in seminal fluid from rabbits varies cyclically and that the length of this cycle is very close to the mean length of the male sexual cycle in this species (Kihlström 1966) as well as to the known length of the male sexual cycle of this particular individual (Degerman and Kihlström 1968).

Furthermore there is a statistical relationship between the seminal volumes which are known to vary cyclically (Kihlström 1966) and the crystallization pattern. Thus, volumes obtained on the first day during periods with exclusively crystals of type R are significantly smaller than the volumes obtained on the most central day during such periods (mean values 0.46 ml and 0.56 ml respectively  $t=2.44$   $f=44$   $p<0.01$ ). Volumes obtained on

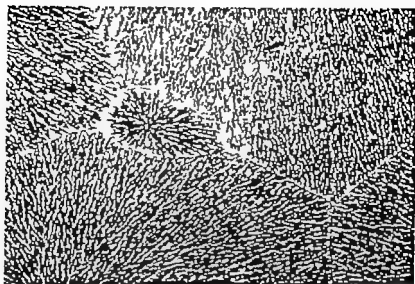


Fig 4 Crystals of type A arranged in a polygonal pattern  
Magnification 1030  $\times$



Fig 5 Crystals transitional between type R and A  
Magnification 1030  $\times$

Table I. The Observed Distributions of the Intervals Between Days with Different Types of Crystals Compared with the Theoretical Distribution to be Found in a Series of Random Numbers

Crystal Pattern	Intervals in Days	Number of Intervals Observed	Number of Intervals to be Found in the Theoretical Distribution	
Crystals of type A	3	10	14.0	$\chi^2 = 6.59$
	4	9	11.7	$p < 0.025$
	5	7	9.3	Mean length:
	6	5		4.7
	7	0		
	8	3		
	11	1		
The polygonal pattern of crystals of type A	3	3	11.2	$\chi^2 = 33.22$
	4	4	9.3	$p < 0.0005$
	5	7	7.5	Mean length:
	6	7		5.8 days
	7	3		
	8	3		
	16	1		
Crystals of type R	3	5	9.2	$\chi^2 = 13.93$
	4	4	7.7	$p < 0.0005$
	5	2	6.1	Mean length:
	6	4		6.6
	7	1		
	8	5		
	13	1		
	27	1		

the first day following upon such periods are also smaller than those obtained on the most central day (mean values 0.49 ml and 0.56 ml respectively  $t = 1.80$   $f = 57$   $p < 0.05$ ). The conclusion must be that the seminal volume increases up to a maximum and begins to decrease again during periods with exclusively crystals of type R. Moreover minimum values of the seminal volumes occur more often than theoretically expected 24 hours after a maximum of the crystals of type A ( $\chi^2 = 5.52$   $p < 0.025$ ).

These facts strongly indicate that the cyclic variation in the crystal pattern of the dried seminal fluid is a further expression of the male sexual cycle previously observed (Kihlström 1966).



Fig. 6 Microphotographs showing crystallization patterns during five successive days (February 25th to March 1st) Magnification 1030  $\times$

## STUDIES ON THE HUMAN PLACENTA

### III. Vascularization of the Young Foetal Placenta

#### A. Vascularization of the Chorionic Villus

BY

FINN BØE

#### *Introduction*

In the preceding study (Bøe 1968) three types of structures were described

- 1 Oblong structures of uniform diameter or slightly swollen, studded with slim projections. These structures constitute the majority of the villi, i.e. the main type.
- 2 Markedly swollen, translucent (oedematous) villi with smooth surface, i.e. without projections.
- 3 Thin, threadlike syncytial bands, likewise, without projections.

Numerous transitional forms occur between the first (main) type and the two remaining secondary types.

The present paper gives a description of the vascularization of these anatomical structures, the relationship of the foetal vasculature to the cell islands and the basal plate and the vascularization of the terminal ramifications of the chorionic tree i.e. of the terminal branches from which the majority of villi arise.

Because different problems are involved, it was found convenient to describe and discuss the material available in two different parts

- A Vascularization of the chorionic villus.
- B Vascularization of the translucent villus and the syncytial band, with special reference to the cell islands and the basal plate.

- Bergman P. *Acta obst. et gynec. scandinav. Suppl.* 4 29 1950  
 Campo De Paz A. *Am J Obst. & Gynec. Suppl* 61 A 190 1951  
 Degerman G. and Kihlström J. E. *Acta physiol. scandinav* 51 108, 1951  
 - *Acta physiol. scandinav* 62 46 1954  
 - Unpublished results 1958  
 Doggett V. C. *Am. J. Physiol.* 187 445 1956  
 Doggett V. C. and Keilers R. K. *Anat. Record* 142 227 1962  
 Exley D. and Corker C. S. *Biochem. J.* 95 54P 1965  
 Harris G. W. *Endocrinol.*, 75 627 1964  
 Hornstein O. Personal communication 1955  
 Hornstein O. Kihlström J. E. and Degerman G. *Acta Endocrinol* 46 608 1964  
 Inoue S. *Zool. Mag. Tokyo* 73 322, 1955a  
 - *Gunna Symp. Endocrinol.* 2 79 1965b  
 Hermack W. O. and McHendrick A. G. *Proc. Roy. Soc. Edinb.* B 57 228 1937  
 Kihlström J. E. Studies on some activities of the male accessory glands, especially the production of male sperm antagghutin and their relations to fertility. Uppsala 1958  
 - *Arkiv Zool.* 15 359 1962  
 - *Acta physiol. scandinav* 59 370 1963  
 - *Acta physiol. scandinav* 65 61 1955  
 - *Experientia* 22 630 1966  
 Kihlström J. E. and Hornstein O. *Acta Endocrinol* 46 597 1964  
 Kihlström J. E. and Hultén C. A. In manuscript 1968  
 Kihlström J. E. and Järnebrant L. *Acta physiol. scandinav* in press 1959  
 Månsson J. C. *Life Science* 4 329 1955  
 Papanicolaou G. N. *Am. J. Anat. Suppl* 52 519 1933  
 Rodrigue-Villa L. *Rev. cubana de lab. clín.* 9 73 1956  
 - *Internat. J. Fertil.* 4 42, 1958  
 Rydberg, E. *Acta obst. et gynec. scandinav* 28 172, 1948  
 Varrapetov B. A. and Demtjenko A. N. *Problemy endokrinology i hormonoterapi* 3 42, 1955  
 Yamane J. *J. Coll. Agric. Hokkaido Imp. Uni.* 19 161 1921  
 Zondek B. *Rec. Progr. Horm. Res.* 10 39 1954

Received on March 19 1958

## STUDIES ON THE HUMAN PLACENTA

### III. Vascularization of the Young Foetal Placenta

#### A. Vascularization of the Chorionic Villus

BY

FINN BÆVRE

#### *Introduction*

In the preceding study (Bævre 1968) three types of structures were described

- 1 Oblong structures of uniform diameter or slightly swollen, studded with slim projections. These structures constitute the majority of the villi, *i.e.* the main type.
2. Markedly swollen, translucent (oedematous) villi with smooth surface, *i.e.* without projections.
- 3 Thin, threadlike syncytial bands, likewise without projections.

Numerous transitional forms occur between the first (main) type and the two remaining secondary types.

The present paper gives a description of the vascularization of these anatomical structures, the relationship of the foetal vasculature to the cell islands and the basal plate and the vascularization of the terminal ramifications of the chorionic tree *i.e.* of the terminal branches from which the majority of villi arise.

Because different problems are involved, it was found convenient to describe and discuss the material available in two different parts

- A. Vascularization of the chorionic villus.
- B Vascularization of the translucent villus and the syncytial band, with special reference to the cell islands and the basal plate.

*Material and technique* in the present study were similar to those described previously. Fresh placentae, removed by hysterotomy were placed in tepid saline solution. The umbilical cord was cut about 3 cm from its insertion and blood forced out by repeated careful pressure. Indian Ink was used as contrast medium and injected into the umbilical artery. In some cases retrograde filling of the vascular system was carried out by injection of contrast medium into the umbilical vein.—The injection should be carried out slowly and at low pressure because of the high fragility of the foetal capillaries. Even a moderate elevation of the intracapillary pressure proves sufficient to provoke rupture of the capillary walls with the formation of extravasates in the villous stroma.—The dissection method and the treatment of the specimens selected for study were described in the two preceding papers (Boe 1967 1968)

### Results

#### 1 *Vascularization of the Minor Chorionic Structures (Syncytial Buds and Projections)*

The paravascular net (PVN Boe 1953) is a system of anastomosing vessels of capillary size which constitutes a system of arterio-venous communications (shunts) between the afferent arteries and the efferent veins of the terminal branches and the villi. The PVN appears mainly and constantly in the terminal branches and the villi of the main type (Figs. 1 and 2)

The net is sited characteristically subjacent to the trophoblastic epithelium lining the latter like a tapestry (Figs. 3 and 4). Therefore, the net might equally well be termed the subepithelial (subtrophoblastic) net and is a two-dimensional structure. Anastomosing capillaries do not appear in the remaining stroma

In the mature placenta the minor chorionic structures (buds and projections) are vascularized from the PVN exclusively (Boe 1953). In the young placenta, likewise the minor chorionic structures are vascularized from the PVN (Figs. 1 and 2). However in these structures separate vessels are not demon-

My original suggestion was the paravascular capillary network



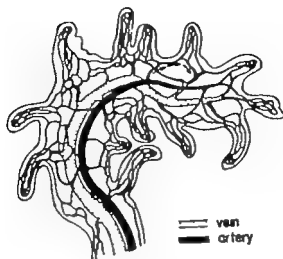


Fig 1 Vascularization of chorionic villus. Schematic drawing. The projections are vascularized from the paravascular net. Arrow indicate shunt mechanism



Fig 2 Chorionic villus, main vessels and PVN well filled with contrast medium. Short projections (above) vascularized from the PVN. 12 weeks. Transillumination 170

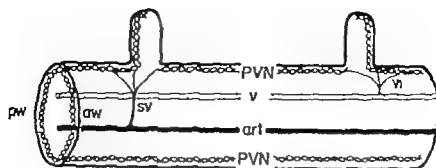


Fig 3. Longitudinal section of a chorionic villus. Schematic drawing. One projection vascularized from the paravascular net (PVN)

art: artery

pw: posterior wall

v: vein

sv: supply vessels

aw: anterior wall

vi: venous inlet



Fig 4 Cut surface of a chorionic villus PVN in the anterior wall in focus (below) appearing blurred in the posterior wall (above) One main vessel level with the PVN (above right) Remaining stroma depleted of capillaries 9 weeks Transillumination  $\times 500$

strable, their supply vessels being indistinguishable in size from the capillaries lining the epithelium. For that reason the PVN will give the impression of continuing unbrokenly into these structures, whose remaining stroma appears devoid of vessels. Like the PVN the position of these capillaries is subepithelial

Since 1953 the PVN has been described by Mayer *et al.* (1956) Crawford (the pre-villous plexus 1961) Arts (1961) Alvarez (1964) Aladjem (1967) and by Cibils (1967)

The supply vessels of the PVN arise from the main vessels of the terminal branches and the villi (Figs. 1 and 3). The supply vessels are of pre-capillary or capillary size. The vessels usually ramify their branches following a diverging course until they split up in the PVN. The arterial (afferent) branches supplying the PVN and the venous (efferent) branches draining the net, exhibit a very similar pattern in the young placenta. The vessels arise at irregular intervals and in every plane, apparently independent of the position of the minor chorionic structures which are vascularized from the PVN. The venous inlets seem to be more numerous than are the arterial supply vessels.—Anastomoses were not observed in the supply vessels.

## 2. Vascularization of the Terminal Ramifications

The minor chorionic structures are vascularized from the PVN. Their supply vessels are indistinguishable in size from the vessels of the net.

A villus may be defined as a chorionic structure with separate supply vessels. These vessels are distinguishable from the vessels of the PVN and the capillaries by their larger size.

A terminal branch may be defined as a ramification of the chorionic tree from which villi arise. The separate vessels of the villus arise from the main vessels of the terminal branch.

The superficial PVN in the terminal branches communicates with the PVN in the villi, the latter continuing unbrokenly into the capillaries of their buds and projections. Obviously the PVN and the capillaries constitute an anastomosing continuum, an anatomical entity. A villus may simply be regarded as a big projection, with additional separate supply vessels. The capillaries of the projections may be regarded an extension of the PVN.

The numerous communications (syncytial bands) between the villi were never entirely vascularized. The PVN thus, represents the only vascular connection between the villi. The chorionic villus, therefore must be regarded as a vasculatory unit (Bes

1953) In this view Crawford (1956 a, b) Arts (1961) and Boyd and Hamilton (1967) agree.

*The main vessels* There are two vessels in every villus and terminal branch, one artery and one vein. The course of the vessels shows a somewhat different pattern in the different villi and branches. The two vessels may run fairly parallel though separated more than in the big vessel-conveying stems. The artery usually follows a more superficial course than does the vein, and the vessels may cross—Very frequently the vessels show a diverging course gradually approaching the PVN artery and vein diametrically opposed until the vessels terminally split up in the apical part of the PVN—In other specimens the vessels may run nearly their entire course superficially close beneath the PVN (Fig 4)—Finally and not infrequently the vessels run in the same plan as the PVN In the latter specimens the remaining stroma appears completely empty of vessels Tortuosity of vessels was not observed.

It appears from the preceding description, that the vascularization of the chorionic structures is less complicated in the young placenta than it is in the mature organ. The different pattern of arteries and veins so characteristic of placental maturity (Boe 1953) could hardly be traced in the young placenta.—The sinusoidal expansions of the capillaries could not be demonstrated in the capillaries of the young placenta.

### *Discussion*

The PVN and the capillaries may be regarded as an anatomical entity the capillaries of the projections being an extension of the PVN The projections greatly increase the surface of the syncytium, and, accordingly facilitate the exchange of metabolites between the two circulations

The anatomical structure of the PVN is characteristic of a shunt mechanism allowing blood to flow directly from the arterial to the venous side of the system eventually by-passing the projections. In this way the PVN will act as a buffer system for the regulation of the haemodynamics in the villi. This mechanism may seem necessary because the chorionic villus must be re-

garded a circulatory unit, the PVN constituting the only vascular link between the projections (and between the villi)

Like the PVN the anatomical position of the capillaries is subepithelial, two-dimensional, lining the inside of the syncytium like a tapestry as close as possible to the maternal blood in the IVS. This is in accord with the function of the capillaries. Presumably the entire capillary system (the PVN included) is concerned solely with the exchange of gases and metabolites between the maternal and the foetal circulations. Accordingly the *enabling stroma* appears devoid of anastomosing capillaries.

Presumably a gradual fall in the blood pressure in the entire vascular system, seems necessary to circulation in the structures.

The evidence presented in this study may be explained by considering the chorionic tree as a structure in continual growth. Consequently sharp distinction between the different chorionic structures does not appear.

### SUMMARY

- 1 The terminal branches, from which the majority of villi arise represent the terminal ramifications of the chorionic tree.
- 2 The main (stem) vessels of the terminal branches supply the villi with their separate vessels.
- 3 The paravascular net is a system of anastomosing vessels of capillary size, interposed between the artery and the vein (main vessels) in the terminal branches and the villi. The latter vessels supply the net with branches of pre-capillary or capillary size.
- 4 The paravascular net continues unbrokenly into the anastomosing capillaries of the syncytial buds and projections. Together the two vascular systems form a continuum, an anatomical entity of which the capillaries of the projections are an extension.
- 5 The anatomical position of this continuum is subepithelial closely beneath and adjacent to the trophoblastic epithelium. Thus, the structure is essentially two-dimensional.
- 6 The chorionic villus is considered as a circulatory unit. The paravascular net represents the only vascular communication between the projections and between the villi.

## REFERENCES

- Aladjem S* Am J Obst. Gynec. 99 350 1967  
*Alvarez H* Obstet. Gynec. 23 813 1964  
*Arts N F Th* Am. J Obst. Gynec. 82 147 1961  
*Boyd J D and Hamilton W J J* Obstet. Gynaec. Brit. Cwlth. 74 161 1967  
*Boe F* Acta obst. et gynec. scandinav 32 suppl. 5 1953  
- Ibidem. 46 591 1967  
- Ibidem. 47 420 1968  
*Cibils L A* Rev franc. Gynec. 62 349 1967  
*Crawford J AL* J Obstet. Gynaec. Brit. Emp 63 87 1956 a  
- Ibidem. 63 542, 1956 b  
- Ibidem 63 378 1961  
*Mayer M Parigel M and Leclerc Polyak H* Gynec. et obst. 55 257 1956

Received on March 15 1968

## STUDIES ON THE HUMAN PLACENTA

### III. Vascularization of the Young Foetal Placenta

#### B. Vascularization of the Translucent Villus and the Syncytial Band, with Special Reference to the Cell Islands and the Basal Plate

BY

FINN BØE

##### 1. *The Translucent Villus*

The extreme type, which is of cucumber form with a smooth surface is an avascular structure, i.e. the vascular system apparently has disintegrated. The different stages in this retrogressive process may be observed in the transitional forms, from the fully vascularized main type studded with slim projections, to the avascular type without projections. The latter type and the transitional forms occur everywhere in the intervillous space (IVS). The majority however are in close relationship to the cell islands and to the basal plate.

In this retrogressive process two features are almost constant,

1. the process starts in the apical part of the villus and the terminal branch,
2. the system of capillaries i.e. the paravascular net (PVN) and the capillaries of the projections, disintegrate before the main vessels do.

##### a. *The system of capillaries*

In several well vascularized villi, swollen, bulb-shaped, translucent projections appear scattered between slim projections (Bøe 1968). The swollen villi show a predominance of bulb-shaped



Fig 1 Villus with plump projections. Three lateral projections (below) arrows indicate the border of another three more centrally placed projections.—Coarse and fine vascular pattern. Areas of projections non-vascularized. (Two photographs taken at slightly different levels are put together) 9 weeks. Transillumination.  $\times 84$

projections (Fig 1) with short, expanded capillaries connected by tiny filaments. The capillaries are irregularly distributed at the base or over the surface of the projections. Areas of the surfaces are devoid of vessels to a varying degree (Fig 1) —When projections are close together apical expansions may assume a pattern resembling of a garland.

On the surface of the swollen villus very tiny projections of varying length are observed. These microprojections may be of uniform diameter or they may terminate like a knot forming a drumstick like structure. In a third variety the upper part of a bulb-shaped projection appears constricted into a tiny protrusion. Invariably these structures are avascular or very poorly vascularized (Fig 2)

In the swollen villi with plump (wartlike) projections similar changes are observed in the PVN (Fig 3) —In advanced changes the capillary system having assumed a uniformly tiny filiform appearance.—When the retrogression is still more advanced, only remnants of the system appear forming scattered, tiny filaments.





Fig. 2. Swollen villi with microprojections—drumstick-shaped one (lower arrow) and one tiny protrusion protruding from the top of plump projection (upper arrow). Several other fine projections are seen. Above, extreme left avascular smooth villus from neighbouring branch. 9 weeks. Dark field.  $\times 66$

—In the extreme type of translucent villus the entire capillary system has disintegrated (Fig. 4 A B C)

When contrast medium is injected into the arterial system and appears in the venous system, the medium, obviously must have passed *via* the capillary system (*vice versa* if the medium was injected into the venous system). The preservation of capillaries, therefore, is a necessary condition for the filling of the opposite vessel with medium. Even when the major part of the capillary system has disintegrated, intact vessels in a limited area prove sufficient to allow passage of contrast medium into the opposite vessel (Figs. 3 and 7). The passage of medium occurs even with advanced, uniform capillary retrogression, *i.e.* in the stage of filiform appearance. However when disintegration has proceeded to a stage where the capillaries are no longer communicating,



Fig. 1 Villus with plump projections. Three lateral projections (below) arrows indicate the border of another three, more centrally placed projections.—Coarse and fine vascular pattern. Areas of projections non-vascularized. (Two photographs taken at slightly different levels are put together) 9 weeks Transillumination.  $\times 84$

projections (Fig 1) with short, expanded capillaries connected by tiny filaments. The capillaries are irregularly distributed at the base or over the surface of the projections. Areas of the surfaces are devoid of vessels to a varying degree (Fig 1) —When projections are close together apical expansions may assume a pattern resembling of a garland.

On the surface of the swollen villus very tiny projections of varying length are observed. These microprojections may be of uniform diameter or they may terminate like a knot, forming a drumstick like structure. In a third variety the upper part of a bulb-shaped projection appears constricted into a tiny protrusion. Invariably these structures are avascular or very poorly vascularized (Fig 2)

In the swollen villi, with plump (wartlike) projections similar changes are observed in the PVN (Fig 3) —In advanced changes the capillary system having assumed a uniformly tiny filiform appearance —When the retrogression is still more advanced only remnants of the system appear forming scattered, tiny filaments.



Fig. 2. Swollen villi with microprojections—drumstick-shaped one (lower arrow) and one tiny protrusion protruding from the top of a plump projection (upper arrow). Several other fine projections are seen. — Above, extreme left avascular smooth villus from neighbouring branch. 9 weeks. Dark field 66.

—In the extreme type of translucent villus the entire capillary system has disintegrated (Fig. 4 A B C)

When contrast medium is injected into the arterial system and appears in the venous system, the medium, obviously must have passed *via* the capillary system (*vice versa* if the medium was injected into the venous system). The preservation of capillaries, therefore is a necessary condition for the filling of the opposite vessel with medium. Even when the major part of the capillary system has disintegrated, intact vessels in a limited area prove sufficient to allow passage of contrast medium into the opposite vessel (Figs. 3 and 7). The passage of medium occurs even with advanced, uniform capillary retrogression, *i.e.* in the stage of filiform appearance. However when disintegration has proceeded to a stage where the capillaries are no longer communicating,



Fig. 1 Villus with plump projections. Three lateral projections (below) arrows indicate the border of another three more centrally placed projections.—Coarse and fine vascular pattern. Areas of projections non-vascularized. (Two photographs taken at slightly different levels are put together) 9 weeks Transillumination.  $\times 84$

projections (Fig 1) with short, expanded capillaries connected by tiny filaments. The capillaries are irregularly distributed at the base or over the surface of the projections. Areas of the surfaces are devoid of vessels to a varying degree (Fig 1) —When projections are close together apical expansions may assume a pattern resembling of a garland.

On the surface of the swollen villus very tiny projections of varying length are observed. These microprojections may be of uniform diameter or they may terminate like a knot, forming a drumstick like structure. In a third variety the upper part of a bulb-shaped projection appears constricted into a tiny protrusion. Invariably these structures are avascular or very poorly vascularized (Fig 2).

In the swollen villi, with plump (wartlike) projections similar changes are observed in the PVN (Fig 3) —In advanced changes the capillary system having assumed a uniformly tiny filliform appearance —When the retrogression is still more advanced only remnants of the system appear forming scattered, tiny filaments.



Fig 4 A. Markedly swollen villus with smooth surface. Artery can be followed shadowlike beyond the limit of contrast medium (arrow), terminating in U-formed configuration in the apical part (right). A few filaments visible on the surface. Vein not visible. 10 weeks. Transillumination.  $\times 66$ .



Fig 4 B. Markedly swollen villus with smooth surface. Part of the artery appears shadowlike. Capillaries and vein not visible. 10 weeks. Transillumination. 105

projections the supply vessels from the PVN being indistinguishable in size from the capillaries. In moderate retrogression, i.e. in incipient disintegration of the vascular system, the pattern becomes more apparent than in the fully vascularized structure. The supply vessel arising from the main artery ramifies, giving off branches to the PVN and terminating in vessels supplying the projections (Fig. 5 A). One vessel enters a projection adjacent to the epithelium, forms an apical loop and continues on



Fig 3 Markedly swollen vilus with plump projections. Venous filling. Artery (above) fades away in the neck (arrow) the vessel filled from the ven (below) *via* the capillaries. Coarse and fine pattern of the capillary system. 13 weeks. Transillumination.  $\times 105$

passage of medium naturally becomes impossible and no medium can be seen in the opposite vessel (Fig 4 A B C)

The filling of the opposite vessel *via* the capillary system is limited to the region adjacent to the capillary area through which the contrast medium has passed. The rest of the vessel up to its origin from the main vessel is empty of medium (Figs. 3 and 7) —This common finding seems to exclude retrograde filling, and, likewise filling through arterio-venous anastomoses, which, apart from the PVN are generally supposed to occur very infrequently in the placenta.

#### b *The main vessels and their branches*

In the well vascularized villus the PVN gives the impression of continuing unbroken into the anastomosing capillaries of the

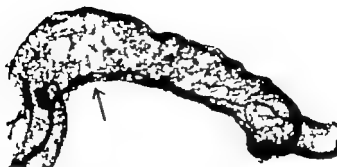


Fig. 4. A. Markedly swollen villus with smooth surface. Artery can be followed shadowlike beyond the limit of contrast medium (arrow), terminating in U-formed configuration in the apical part (right). A few filaments visible on the surface. Vein not visible. 10 weeks. Transillumination.  $\times 65$ .



Fig. 4. B. Markedly swollen villus with smooth surface. Part of the artery appears shadowlike. Capillaries and vein not visible. 10 weeks. Transillumination.  $\times 105$ .

projections, the supply vessels from the PVN being indistinguishable in size from the capillaries. In moderate retrogression, i.e. in incipient disintegration of the vascular system, the pattern becomes more apparent than in the fully vascularized structure. The supply vessel arising from the main artery ramifies giving off branches to the PVN and terminating in vessels supplying the projections (Fig. 3 A). One vessel enters a projection adjacent to the epithelium, forms an apical loop and continues on



Fig 3 Markedly swollen villus with plump projections. Venous filling. Artery (above) fades away in the neck (arrow) the vessel filled from the vein (below) via the capillaries. Coarse and fine pattern of the capillary system. 13 weeks. Transillumination.  $\times 105$

passage of medium naturally becomes impossible and no medium can be seen in the opposite vessel (Fig. 4 A B C)

The filling of the opposite vessel *via* the capillary system is limited to the region adjacent to the capillary area through which the contrast medium has passed. The rest of the vessel, up to its origin from the main vessel is empty of medium (Figs. 3 and 7) —This common finding seems to exclude retrograde filling, and, likewise filling through arterio-venous anastomoses which, apart from the PVN are generally supposed to occur very infrequently in the placenta

#### *b The main vessels and their branches*

In the well vascularized villus the PVN gives the impression of continuing unbroken into the anastomosing capillaries of the





Fig. 5 Swollen villus, moderate regressive changes. A Arterial pattern. Supply vessel from the stem artery (upper arrow) gives off branches to the PVN and terminates in three capillary vessels, one to each of three projections (lower arrows) 12 weeks T small illumination  $\times 111$

mutely fade away shadowlike in apical direction. Probably the reason for the medium failing to penetrate the vessel or penetrating imperfectly giving a shadow of the vessel, is occlusion of the lumen by endothelial hyperplasia, which is a frequent finding in the histological picture. Avascular villi were recently described by Baddai *et al.* (1967).

The small and medium sized stems attached to the basal plate



Fig. 4 C Markedly swollen villus with smooth surface. Artery can be followed shadowlike beyond the limit of contrast medium (arrow) Capillaries and vein not visible. 10 weeks. Transillumination.  $\times 105$

the opposite side as the efferent (venous) capillary. The latter vessel receives tributaries from the PVN draining the net, and joins the main vein (Fig. 5 B)

In markedly swollen villi with plump projections the supply vessels show alternate tiny (contracted) and expanded (dilated) areas (Fig. 6). The main vessels also may appear dilated. Frequently the expansions are most conspicuous in the apical part of the villus (Figs. 1 and 10).—In more advanced cases the dilated capillaries apparently coalesced into a single vessel, uniting the afferent and efferent vessels to form a loop (Fig. 8 A B). When this vascular loop is demonstrable the capillaries have disintegrated, or only remnants are visible. Therefore it is more likely that the expansions are formed by coalescence of capillaries than by dilatation of pre-existing vessels.—In terminal branches as well the vessels may terminate in loop formation (Fig. 8 A).

The main vessels most frequently terminate blindly in the swollen, translucent apical area, the contrast medium fading away. The more the disintegration has proceeded i.e. the nearer the formation of the extreme type the shorter will the vessels appear.—In many specimens the vessels may be clearly visible for some distance beyond the limit of medium, and they ulti

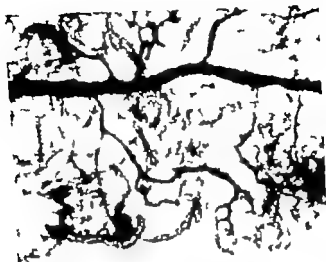


Fig 6 Terminal branch. Main artery on level with the PVN. Tiny supply vessels from the artery show expansions. Coarse (expanded) and fine capillaries in the plump flattened projections areas of which appear non-vascular used 10 weeks Transillumination.  $\times 105$

area. More rarely the artery may run a short distance into the area giving off no branches.—Not infrequently the vessels show a terminal chandelier pattern, bending into the IVS from the border of the translucent area (Fig. 9)

The characteristic swelling of the translucent villus apparently is due to oedema in the villous stroma. The genesis of this oedema is not obvious from the results of the present study. No signs of stasis were apparent. Increased permeability of the capillaries during their disintegration, may be another possibility

## 2. The Syncytial Band

The syncytial bands, forming a framework in the IVS most frequently are avascular structures. Nevertheless, the bands may sometimes appear to be vascularized, but hardly ever in their



Fig 5 ■ Venous pattern (photograph taken from the back of the slide) Tributaries to the main vein (arrows) drain the three projections and receive tributaries from the PVN 12 weeks T anillumination.  $\times 84$

( anchoring villi ) usually are moderately swollen and may appear well vascularized. At their attachment, the structure is moderately swollen and translucent, without projections. When these anchoring villi are detached from the basal plate their apices are covered by a cap of opaque tissue severed from the plate (Fig. 9) —Usually the main vessels fade away or terminate more abruptly on the border of the translucent apical



Fig. 6 Terminal branch. Mat. artery on level with the PVN. Tiny supply vessels from the artery show expansions. Coarse (expanded) and fine capillaries in the plump flattened projections, areas of which appear non-vascular used 10 weeks Transillumination.  $\times 105$

area. More rarely the artery may run a short distance into the area, giving off no branches.—Not infrequently the vessels show a terminal chandelier pattern, bending into the IVS from the border of the translucent area (Fig. 9)

The characteristic swelling of the translucent villus apparently is due to oedema in the villous stroma. The genesis of this oedema is not obvious from the results of the present study. No signs of stasis were apparent. Increased permeability of the capillaries during their disintegration, may be another possibility.

## 2. The Syncytial Band

The syncytial bands forming a framework in the IVS most frequently are avascular structures. Nevertheless, the bands may sometimes appear to be vascularized, but hardly ever in their



Fig 7 Small stem. Venous filling Left: Swollen villus with smooth surface. Vein fades away shadowlike in the apical area. PVN preserved in a limited area in the neck. Contrast medium appears in a fragment of the artery (left arrow) filled via capillaries.—Right: Complex of villi. Main vein fades away inlet of a supply vessel (centre arrow) Filliform appearance of the capillary system. Right arrow Medium in the main artery having passed via the capillaries, terminates abruptly. A supply vessel arises from the artery 13 weeks Dark field  $\times 33$ .

entirety The bands thus do not form vascular communications between the villi

Usually the band is most extensive at its origin most frequently from the apical part of the villus gradually narrowing during its further course into thin smooth threadlike and avascular structures

The syncytial band is vascularized from the main vessels of the villus from which it arises. In some specimens both vessels may be followed for some distance united by remnants of the PVN until fading away Not infrequently the vessels terminate in loop formation. Perhaps most frequently only one vessel appears filled with contrast medium, the PVN and the opposite vessel being absent. Occasionally PVN from the villus may



Fig 8 A. Venous filling. Below: Vascular loop in smooth, swollen terminal branch. Lower arrow: Reflux of contrast medium but the artery terminates; the vessel can be followed shadowlike beyond this point.—Above: Vascular loop in smooth, swollen villus. Upper arrow: Reflux of contrast medium into the artery terminates; the vessel fades away. 9 weeks. Transillumination.

III



Fig 8 B. Vascular loop in a translucent placenta. 9 weeks. Transillumination.

84



Fig. 9 Villous stem (anchoring villus) disrupted from the basal plate. Chandeller pattern of the vessels bending into the IVS from the apical translucent area. Opaque tissue (top left) severed from the basal plate. 9 weeks Transillumination.  $\times 53$

continue for some distance into the band and then fade away — If the short projections are vascularized, they are supplied from the PVN — In each specimen remnants of capillaries may be found near the origin of the band

### *3 Relationship of the Vasculature to the Cell Islands*

The cell islands are avascular structures although vascularized foetal structures frequently occur in close relationship to the surface of the islands.

Terminal branches and even major structures which are filled to a varying degree with contrast medium may be attached to the cell islands by their free, apical part very similar to the attachment of the anchoring villi to the basal plate. Likewise vascularized villi and projections may be observed attached to





Fig. 10. Small villus (above right) attached to cell island, capillaries form tiny loops level with the surface - Vascular loop (expanded) and remnants of capillaries in transplacental villus (left) attached to the island by a short, avascular syncytial band. 9 weeks. Dark field.  $\times 84$ .

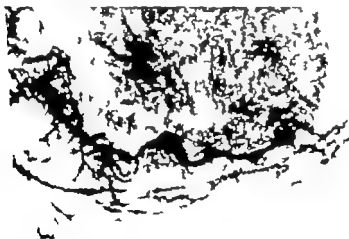


Fig. 11. Transplacental villi attached to cell island on lateral aspect. Vascular expansions (garland pattern) in the main vessel adjacent to the surface. Ordinary calibre and course of the opposite vessel (below), fading away in apical direction (centre right) 9 weeks. Transillumination.  $\times 84$ .

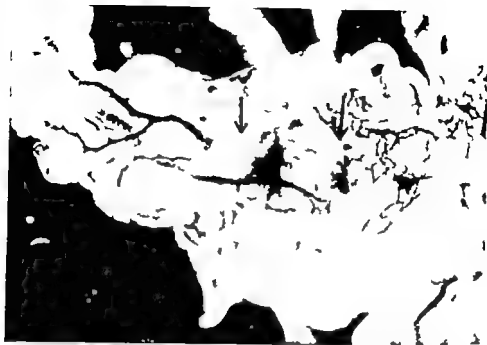


Fig. 12. Translucent villus attached to a cell island on lateral aspect. The apical part (extreme left) is vascularized via main vessels, intimately adjacent to the surface. Note the poor filling of these vessels especially the upper one (arrows) 9 weeks Transillumination  $\times 84$

the islands by their free apical part (Fig. 10) and structures may also appear attached to the islands on their lateral aspect (Figs. 11 and 12)

The degree of vascularization varies considerably. Close to the surface the dilated vessels (garland pattern) predominate (Figs. 11 and 13 A). Level with the surface the adjacent wall of the attached structure apparently disintegrates. The dilated vessels seem to grow out of the structure exhibiting a short radial course. They attach clawlike to the surface (Fig. 13 B).—In other specimens the vessels show a more elongated course thus forming a small corona (Fig. 13 C) the vessels fading away on the surface.—When the structures are attached on their lateral aspect the main vessel adjacent to the surface may exhibit definite expansions (garland pattern) whereas the opposite vessel, being free of the surface shows a more normal calibre and

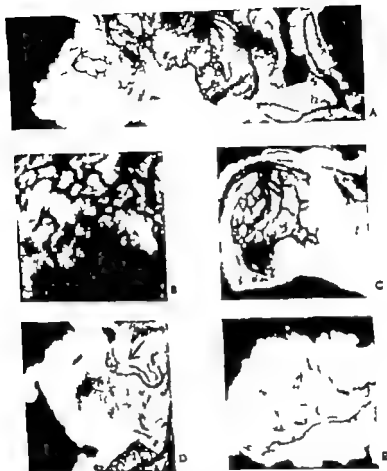


Fig. 13 A: Vascularized structures attached to two cell islands. Right island: Expanded capillaries (garland pattern) Left island: Widespread tiny capillaries level with the surface—Extreme right: Vascular loop in villus attached to the island 9 weeks. Dark field.  $\times 84$  B: Expanded capillaries attach clawlike to the surface of cell island 9 weeks. Dark field.  $\times 105$  C: Small villus attached to cell island. Capillaries grow out of the structure, form small coronas on the surface 9 weeks. Dark field.  $\times 84$  D: Small villus attached to the surface of cell island, afferent and efferent vessels visible. Note the poor vascularization of the vessels level with the surface (arrow) and the richer filling of the projections protruding from the surface (above) 9 weeks. Dark field.  $\times 53$  E: Tiny vessels from attached structure fade away on the surface of cell island 9 weeks. Dark field.  $\times 53$

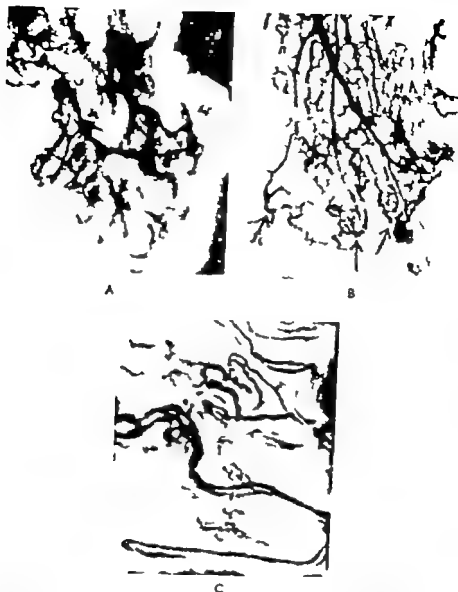


Fig. 14 Vascularized structures attached to the inside of the basal plate. A Expanded esse attach lawlike to the surface. B From the same specimen, area beside that of the preceding one. The esse are more level with the surface. Note tiny supply vessels level with the surface, vascularizing three small groups of projection slightly protruding from the surface (arrows). 9 weeks. Dark field.  $\times 60$ . C Vascular loop formations level with the surface. 13 weeks. Dark field.  $\times 60$ .

course (Fig. 11) When the main vessels are more intimately adjacent to the island, i.e. level with the surface, the vessels appear very poorly filled with contrast medium in this area (Fig. 12) most likely due to endothelial hyperplasia.

When several projections are attached to the surface almost invariably the projections level with the surface are more poorly filled with contrast medium than those protruding from the surface (Fig. 13 D) —The vessels show different patterns, such as a wide-meshed net of capillaries spread over a limited area of the surface (Fig. 13 A) and loop formations (Fig. 10) Frequently isolated vessels may be followed from the attached structure, running for some distance on the surface then fading away (Fig. 13 D E)

The preservation of isolated vessels and capillaries on the surface, seems to be in accord with the previous experience the argyrophilic reticular fibres in the walls of the vessels are more resistant to disintegration than are the collagenous fibres in the stroma (Boe 1967)

#### *4 Relationship of the Vasculature to the Basal Plate*

The similarity of the attachment of the stem villi to the basal plate and the insertion of homologous structures to the cell islands was emphasized previously —The vascularized structures attached to the inside of the basal plate, show the very same characteristics as those attached to the surface of the cell islands vascular expansions, poor vascularization of the structures adjacent to the plate, the formation of vascular loops, and isolated vessels fading away superficially Further description would be repetitive Reference is made to Fig. 14 A B C and to the legends

#### *Discussion*

The two secondary structures, the translucent villus and the syncytial band, are considered derivatives of the main type of villus. The vessels of the latter appear well filled with contrast medium, whereas very little or no medium penetrates into those of the two secondary structures. As judged from the transitional forms,

it is apparent that a marked parallelism exists between the gross transformation of the villus and the disintegration of the vascular system.

The gradual disintegration of the vasculature seems to start in the capillary system. Later the main vessels become involved. The process starts in the apical part of the villus. Accordingly villi with apices translucent and avascular are a frequent finding in the young placenta. In these transitional forms characteristic changes during the vascular disintegration were observed. In the smooth translucent, markedly swollen villus, the entire vascular system has disintegrated the structure thus appearing avascular.

The relationship of the avascular structures to the cell islands and to the basal plate is very similar. Likewise, all essential characteristics of the vascularised structures at their attachment to the surface of the cell islands and to the inside of the basal plate were similar.

The growth of the chorionic tree occurs in the direction of the basal plate and the cell islands. In these structures disintegrated tissue seems to become absorbed.—New branches grow out from pre-existing branches, replacing the decayed structures. Thus, growth and decay are running parallel.

The young placenta is a rapidly growing organ, in which continual development and differentiation occur. The placental metabolism is extraordinary because the foetal placenta is nourished from the maternal organism, *i.e.* from an organism biologically and genetically different from the foetus. The material necessary for the biosynthesis of the foetal structures (anabolic processes) is available in the maternal blood conveying the material to the foetal placenta.

In what way does the placenta discharge the degradation products *i.e.* the result of the catabolic processes? This is the underlying main problem in the present and the two preceding papers. My hypothesis may be presented as follows. The translucent villus and the syncytial band are avascular consequently nonfunctioning structures being regarded as worn-out tissues. Obviously before these structures can be transported by the maternal blood the structures must undergo degradative processes resulting in the formation of substances of low molecular

size. Such waste products will be able to pass into the maternal circulation, from where they are excreted after further degradation if necessary.

These degradative processes particularly occur in the cell islands and the basal plate. The avascular structures are gradually transformed into structureless, amorphous fibrinoid (presumably insoluble protein) revealed grossly by increasing opacity of the cell islands (Bee 1967). The maternal enzyme systems cause the breakdown of fibrinoid into low molecular weight waste products.

This hypothesis may be supported by the evidence presented in the present and the two above mentioned papers. Conclusive evidence, however, may be available when the enzyme systems in question, and the different steps in the degradation process are identified by biochemistry.

### SUMMARY

1. The fully vascularized main type of villus with numerous projections, becomes transformed into two secondary avascular structures, the translucent villus and the syncytial band, both structures without projections.
2. The different stages in the progressive disintegration of the vascular system were observed in the transitional forms.
3. A marked parallelism exists between the transformation of the villus, and the disintegration of the vascular system.
4. The disintegration starts in the apical part of the villus and the terminal branch.
5. The system of capillaries disintegrates before the main vessels.
6. In moderate transformation of the villus, short capillary expansions appear. In advanced changes the capillary system assumes a uniformly tiny filiform appearance.
7. In the translucent apical part of the villus the main vessels fade away or terminate more abruptly, not infrequently in the formation of a vascular loop.
8. Foetal vessels were not observed inside the area of the cell

it is apparent that a marked parallelism exists between the gross transformation of the villus and the disintegration of the vascular system.

The gradual disintegration of the vasculature seems to start in the capillary system. Later the main vessels become involved. The process starts in the apical part of the villus. Accordingly villi with apices translucent and avascular are a frequent finding in the young placenta. In these transitional forms characteristic changes during the vascular disintegration were observed. In the smooth translucent, markedly swollen villus the entire vascular system has disintegrated, the structure thus appearing avascular.

The relationship of the avascular structures to the cell islands and to the basal plate is very similar. Likewise all essential characteristics of the vascularized structures at their attachment to the surface of the cell islands and to the inside of the basal plate were similar.

The growth of the chorionic tree occurs in the direction of the basal plate and the cell islands. In these structures disintegrated tissue seems to become absorbed.—New branches grow out from pre-existing branches replacing the decayed structures. Thus, growth and decay are running parallel.

The young placenta is a rapidly growing organ, in which continual development and differentiation occur. The placental metabolism is extraordinary because the foetal placenta is nourished from the maternal organism, i.e. from an organism biologically and genetically different from the foetus. The material necessary for the biosynthesis of the foetal structures (anabolic processes) is available in the maternal blood, conveying the material to the foetal placenta.

In what way does the placenta discharge the degradation products i.e. the result of the catabolic processes? This is the underlying main problem in the present and the two preceding papers. My hypothesis may be presented as follows. The translucent villus and the syncytial band are avascular consequently nonfunctioning structures being regarded as worn-out tissues. Obviously before these structures can be transported by the maternal blood the structures must undergo degradative processes resulting in the formation of substances of low molecular



From the Department of Obstetrics and Gynaecology (Professor A. Ingelman-Sundberg) Sabbatsbergs Sjukhus and the Department of Pediatrics (Professor R. Zetterström) Kronprinsessans Lovisas Barnsjukhus Stockholm Sweden

## DIETARY HABITS DURING PREGNANCY

A Pilot Study

BY

N.-O. LUNELL, B. PERSSON AND G. STERKY

Our knowledge of the nutritional requirements during normal pregnancy is limited (WHO 1965). An important part of the management of pregnant women with different stages of diabetes mellitus is appropriate dietary advice. To determine the need for nutritional instruction and supervision in normal pregnancy a better insight into the diets of pregnant women seemed necessary.

### *Material and Methods*

Fifteen women were interviewed once during each trimester (group I). Their mean weight before pregnancy was 59.1 kg (44–70 kg). Their mean weight increase during pregnancy was 11.9 kg (7–17 kg). All but one were delivered between the 38th and 42nd week of pregnancy. The birth weights and lengths of all infants fell within  $\pm 1$  S.D. of the normal values given by Engström and Sterky 1966. Five women were between 19 and 21 years, three 24–26 years, four 27–28 years and three above 30 years of age.

Fifty-eight women were interviewed once during pregnancy (group II). Thirty-eight were primigravidae and twenty multigravidae. The mean weight before pregnancy was 56.6 kg (45–78 kg) and the mean weight increase was 12.1 kg (5.8–21.0 kg). All but one was delivered after a normal gestational period and only in four cases did birth weight and foetal length fall outside  $\pm 2$  S.D. of normal values.

The physical activity or possible changes of activity of the

islands and the basal plate. The majority of villi and syncytial bands attached to these structures were avascular.

- In the vascularized foetal structures attached to the surface of the cell islands, vascular expansions, vascular loop formations, isolated capillaries, and vessels fading away on the surface were observed. Very similar changes appeared in the vascularized foetal structures attached to the inside of the basal plate.

### *Acknowledgements*

The author owes a debt of a special gratitude to Dr Elizabeth M. Ramsey whose untiring interest and helpful criticism through several years have been of vital importance for the publication of this and the preceding papers.

The studies were supported by grants from *Norges Almenvitenskapelige Forskningsråd*.

### REFERENCES

- Bakkara L, Gavrilita L, Mardaras A, N. Tomosolu D and Iurgu J. *Am J Obst. Gynec.* 97: 257, 1967.  
 Boe F., *Acta obst. et gynec. scandinav.* 46: 591, 1967.  
 - *Ibidem.* 47: 420, 1968.

Received on March 15, 1968

From the Department of Obstetrics and Gynaecology (Professor A. Ingelman-Sundberg) Sabbatsberga Sjukhus and the Department of Pediatrics (Professor R. Zetterström) Krouphussenska Lörssa Barnsjukhus Stockholm Sweden

## DIETARY HABITS DURING PREGNANCY

A Pilot Study

BY

N.-O. LUNELL, B. PERSSON AND G. STERKY

Our knowledge of the nutritional requirements during normal pregnancy is limited (WHO 1965). An important part of the management of pregnant women with different stages of diabetes mellitus is appropriate dietary advice. To determine the need for nutritional instruction and supervision in normal pregnancy a better insight into the diets of pregnant women seemed necessary.

### *Material and Methods*

Fifteen women were interviewed once during each trimester (group I). Their mean weight before pregnancy was 59.1 kg (44–70 kg). Their mean weight increase during pregnancy was 11.9 kg (7–17 kg). All but one were delivered between the 38th and 42nd week of pregnancy. The birth weights and lengths of all infants fell within  $\pm 1$  S.D. of the normal values given by Engström and Sterky 1966. Five women were between 19 and 21 years, three 24–26 years, four 27–28 years and three above 30 years of age.

Fifty-eight women were interviewed once during pregnancy (group II). Thirty-eight were primigravidas and twenty multigravidas. The mean weight before pregnancy was 56.5 kg (45–78 kg) and the mean weight increase was 12.1 kg (5.8–21.0 kg). All but one was delivered after a normal gestational period and only in four cases did birth weight and foetal length fall outside  $\pm 2$  S.D. of normal values.

The physical activity or possible changes of activity of the

Table I. Mean intake of Calories and Nutrients and Percentage of Recommended Standards in 15 Pregnant Women (Group I) Interviewed During Each Trimester

Duration of Pregnancy	< 16 w		16-28 w		> 28 w	
Calories	1980	94 %	2396	104 %	2085	91 %
Protein g	65	111 %	87	111 %	72	93 %
Fat g	95	121 %	116	134 %	90	105 %
Carbohydrates g	202	73 %	234	81 %	233	91 %
Calcium mg	953	119 %	1331	102 %	1292	99 %
Phosphorous mg	1256	-	1754	-	1504	-
Iron mg	12.8	85 %	18.6	93 %	12.0	60 %
Vitamin A IU	1935	65 %	4489	124 %	2249	62 %
Vitamin D µg	5.1	-	5.1	51 %	0.6	5 %
Vitamin E mg	7.3	-	9.7	-	7.5	-
Thiamine mg	1.3	144 %	1.6	160 %	1.3	128 %
Riboflavin mg	1.7	131 %	2.4	152 %	2.2	135 %
Niacin mg	10.7	76 %	14.7	86 %	11.5	68 %
Ascorbic acid mg	66.0	94 %	147.0	147 %	142.0	142 %

Table II. Mean Intake of Calories and Nutrients and Percentage of Recommended Standards in 58 Pregnant Women (Group II) Each Interviewed Once During Pregnancy (Number of Women in Brackets)

Duration of Pregnancy	< 16 w (18)		16-28 w (13)		> 28 w (27)	
Calories	2035	95 %	2185	92 %	2137	91 %
Protein g	66	114 %	70	90 %	73	93 %
Fat g	98	123 %	104	118 %	92	105 %
Carbohydrates g	209	73 %	224	75 %	245	83 %
Calcium mg	1009	106 %	1163	78 %	107	91 %
Phosphorous mg	1292	-	1405	-	1533	-
Iron mg	13.6	83 %	13.4	64 %	14.2	71 %
Vitamin A IU	2629	89 %	2278	63 %	2257	63 %
Vitamin D µg	2.5	-	3.6	36 %	2.3	23 %
Vitamin E mg	9.6	-	11.5	-	8.8	-
Thiamine mg	1.3	153 %	1.2	118 %	1.5	142 %
Riboflavin mg	1.7	130 %	1.8	111 %	2.2	135 %
Niacin mg	11.5	81 %	10.2	60 %	10.6	62 %
Ascorbic acid mg	91.0	129 %	103.0	103 %	116.0	116 %

Table III Mean (number of Calories and Nutrients) (Group II) Correlated: Age  
(Number of Women in Brackets)

Age	17-19 Years (11)	20-24 Years (27)	25-29 Years (15)	30-40 Years (5)
Calories	1851	2321	1836	2160
Protein g	66	75	64	74
Fat g	87	110	79	83
Carbohydrates g	214	242	206	263
Calcium mg	1168	1223	1222	881
Phosphorus mg	1359	1307	1384	1305
Iron mg	11.9	14.4	10.9	19.5
Vitamin A I.U.	1649	2936	2233	1754
Vitamin D $\mu$ g	2.1	3.1	2.5	2.2
Vitamin E mg	8.4	10.7	9.1	8.4
Thiamine mg	1.4	1.5	1.1	1.3
Riboflavin mg	1.8	2.1	1.9	1.6
Niacin mg	10.3	12.6	7.7	11.7
Ascorbic acid mg	96.0	120.0	68.0	157.0
				160.0

Table I. Mean Intake of Calories and Nutrients and Percentage of Recommended Standards in 15 Pregnant Women (Group I) Interviewed During Each Trimester

Duration of Pregnancy	< 16 w		16-28 w		> 28 w	
Calories	1960	94 %	2396	104 %	2083	91 %
Protein g	65	111 %	87	111 %	72	93 %
Fat g	95	121 %	116	134 %	90	105 %
Carbohydrates g	202	73 %	234	81 %	233	81 %
Calcium mg	953	119 %	1331	102 %	1292	99 %
Phosphorous mg	1256	-	1754	-	1504	-
Iron mg	12.8	85 %	18.6	93 %	12.0	60 %
Vitamin A LU	1936	65 %	4489	124 %	2249	62 %
Vitamin D µg	5.1	-	5.1	51 %	6	26 %
Vitamin E mg	7.3	-	9.7	-	7.5	-
Thiamine mg	1.3	144 %	1.6	160 %	1.3	126 %
Riboflavin mg	1.7	131 %	2.4	152 %	2.2	135 %
Niacin mg	10.7	76 %	14.7	86 %	11.5	68 %
Ascorbic acid mg	66.0	94 %	147.0	147 %	142.0	142 %

Table II. Mean Intake of Calories and Nutrients and Percentage of Recommended Standards in 58 Pregnant Women (Group II) Each Interviewed Once During Pregnancy (Number of Women in Brackets)

Duration of Pregnancy	< 16 w (18)		16-28 w (13)		> 28 w (27)	
Calories	2035	95 %	2185	92 %	2137	91 %
Protein g	66	114 %	70	90 %	73	93 %
Fat g	98	123 %	104	118 %	92	106 %
Carbohydrates g	209	73 %	224	75 %	245	83 %
Calcium mg	1009	106 %	1168	78 %	1207	91 %
Phosphorous mg	1292	-	1405	-	1533	-
Iron mg	13.6	83 %	13.4	64 %	14.2	71 %
Vitamin A LU	2629	69 %	2278	63 %	2287	63 %
Vitamin D µg	2.5	-	3.6	36 %	2.3	23 %
Vitamin E mg	9.6	-	11.5	-	8.8	-
Thiamine mg	1.3	153 %	1.2	118 %	1.5	142 %
Riboflavin mg	1.7	130 %	1.8	111 %	2.2	135 %
Niacin mg	11.5	81 %	10.2	60 %	10.6	62 %
Ascorbic acid mg	91.0	129 %	103.0	103 %	116.0	116 %

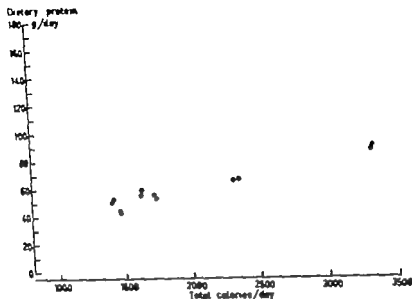


Fig 1 The relation between intake of calories and protein in individual cases

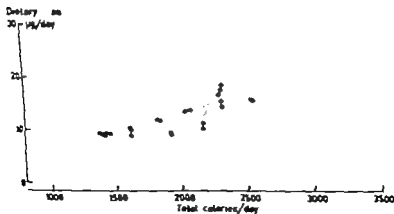


Fig 2 The relation between intake of calories and iron in individual cases

Table IV Mean Nutritional Intake of Married and Unmarried Pregnant Women (Number in Brackets) From Group I Individual Mean Values Are Used

Group I + II (73)		Calories	Protein g	Iron mg	Calcium mg
Unmarried	17-19 years (8)	2074	0	13.4	10.9
Unmarried	20-29 years (10)	2322	9	1.4	12.5
Married	17-19 years (5)	1908	67	10.4	1090
Married	20-40 years (50)	2102	70	13.6	11.1

Table V Mean Nutritional Intake of Primigravidae and Multigravidae (Group I + II) From Group I Individual Mean Values Are Used

		Calories	Protein g	Iron mg	Calcium mg
Primigravidae	(47)	2169	72	13.5	11.8
Multigravidae	(26)	2032	69	13.8	11.35

pregnant women were not known at the interviews. The dietary intake was calculated using the 24-hour recall method (for details see Sterky 1962). Food consumption tables compiled by Abramson 1960 were used for calculating the nutritive values of the various food-stuffs consumed. In order to make a comparison possible all results are given as percentages of recommended standards (Abramson 1960). According to these recommendations a supplement of 200 calories and increases of certain nutrients are added after the second trimester.

### Results

In Table I the mean intake of calories and nutrients for group I is given. Corresponding values for group II are given in Table II. Table III shows the mean intake in relation to age. Table IV gives the nutritional intake of married and unmarried women and Table V that of primigravidae and multigravidae. The correlation between the intake of calories and proteins is given in Fig. 1 and between calories and iron in Fig. 2. The mean intake of protein is 33.7 g and of iron 6.7 mg per 1000 calories.



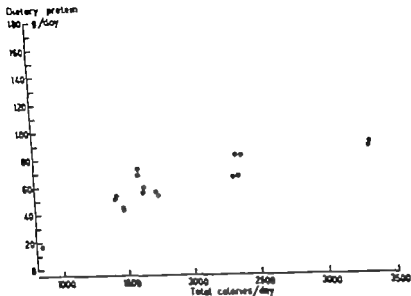


Fig 1 The relation between intake of calories and protein in individual cases

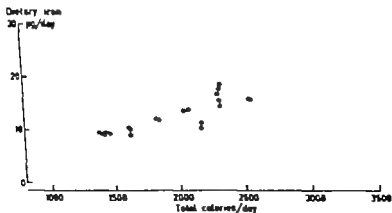


Fig 2 The relation between intake of calories and iron in individual cases

Table IV *Mean Nutritional Intake of Married and Unmarried Pregnant Women (Number in Brackets) From Group I Individual Mean Values Are Used*

Group I+II (73)			Calories	Protein g	Iron mg	Calcium mg
Unmarried	17-19 years	(8)	2074	70	13.4	10.9
Unmarried	20-29 years	(10)	2322	79	15.4	12.6
Married	17-19 years	(5)	1968	67	10.4	10.90
Married	20-40 years	(50)	2102	70	13.6	11.71

Table V *Mean Nutritional Intake of Primigravidae and Multigravidae (Group I+II) From Group I Individual Mean Values Are Used*

		Calories	Protein g	Iron mg	Calcium mg
Primigravidae	(47)	2169	72	13.5	11.78
Multigravidae	(26)	2032	69	13.8	11.36

pregnant women were not known at the interviews. The dietary intake was calculated using the 24-hour recall method (for details see Sterky 1962). Food consumption tables compiled by Abramson 1960 were used for calculating the nutritive values of the various food stuffs consumed. In order to make a comparison possible all results are given as percentages of recommended standards (Abramson 1960). According to these recommendations a supplement of 200 calories and increases of certain nutrients are added after the second trimester.

### Results

In Table I the mean intake of calories and nutrients for group I is given. Corresponding values for group II are given in Table II. Table III shows the mean intake in relation to age. Table IV gives the nutritional intake of married and unmarried women and Table V that of primigravidae and multigravidae. The correlation between the intake of calories and proteins is given in Fig. 1 and between calories and iron in Fig. 2. The mean intake of protein is 33.7 g and of iron 6.7 mg per 1000 calories.

In our patients there was a tendency to a moderate caloric increase between the first and second trimester best seen in group I. A smaller reduction occurred from the second to the third trimester. The increase corresponded approximately to the additional 200 calories included in the recommended standards for pregnancy.

It is noteworthy that in all groups the intake of iron was below the recommendations. The low dietary iron is of further importance in view of the fact that one third of the women did not take their prescribed drugs because of fear of side-effects or injury to the foetus. This is in accordance with the observation of Hallberg (1966) and also as discussed by WHO (1965).

Contrary to the assumption by WHO a deficient intake of calcium could not be demonstrated. Vitamin D and niacin were, besides iron, the only nutrients that invariably were below the recommendations. The intake of ascorbic acid was adequate and, as in other Swedish studies did not correlate with the caloric consumption.

The civil status or number of children did not seem to influence the quality of intake and the choice of food was very constant. Thus, pregnant women with low caloric consumption run the risk of getting an inadequate supply of certain nutrients.

The mean weight increase was in our series, equal to that usually found in normal pregnancy (Lumell 1966). During normal pregnancy there is a considerable storage of fat by the mother (Taggart *et al.* 1967). Whether this can be or ought to be influenced by dietary changes can not be answered in this study.

The results indicate that dietary instructions to pregnant women with special disorders such as diabetes mellitus must be done with great care in order to influence deep-rooted patterns in their choice of food.

## SUMMARY

The dietary pattern of normal pregnant women was analysed using the 24-hour recall method. Fifteen women were followed from the first to the third trimester and fifty-eight were studied

*Table VI. The Percentage Distribution of Calories among Different Foods in Groups I and II*

Dried peas and beans	1.0
Vegetables	1.0
Fruits and berries	6.9
Potatoes and roots	6.0
Milk and cheese	22.2
Meat fish and eggs	21.1
Bread and cereals	20.2
Fats and oils	10.7
Sugar jam and juice	11.2

The distribution of calories among different foods is seen in Table VI. In group I there seemed to be an increase in the consumption of milk products with increasing duration of pregnancy while a corresponding decrease in the intake of meat, fish and eggs occurred.

Group II was questioned about the intake of prescribed iron and vitamins. Eighteen out of forty women had taken iron and vitamins eight only iron, one only vitamins and thirteen women no drugs.

The consumption of calories may be broken down as 14 % protein 42 % fat and 44 % carbohydrates.

### *Comments*

The mean consumption of calories and nutrients in this series was generally satisfactory. The pattern seems to be similar to that among the general population. The correlation between protein and calories and iron and calories (Figs. 1 and 2) is as constant as was earlier found for non-pregnant women (Blitz 1965 Carlgren and Cramér 1965). The mean intake of calories (2100) in our patients was about 200 calories more than that found in 23 pregnant women by Hallberg, 1966. A considerably higher intake was noticed by Stevens and Ohlson 1967 in 129 pregnant women. They found a satisfactory intake of almost all nutrients and a considerable improvement compared with a similar study conducted in the same place (Iowa City USA) in 1952.

In our patients there was a tendency to a moderate caloric increase between the first and second trimester best seen in group I. A smaller reduction occurred from the second to the third trimester. The increase corresponded approximately to the additional 200 calories included in the recommended standards for pregnancy.

It is noteworthy that in all groups the intake of iron was below the recommendations. The low dietary iron is of further importance in view of the fact that one third of the women did not take their prescribed drugs because of fear of side-effects or injury to the foetus. This is in accordance with the observation of Hallberg (1966) and also as discussed by WHO (1965).

Contrary to the assumption by WHO a deficient intake of calcium could not be demonstrated. Vitamin D and niacin were, besides iron, the only nutrients that invariably were below the recommendations. The intake of ascorbic acid was adequate and, as in other Swedish studies, did not correlate with the caloric consumption.

The civil status or number of children did not seem to influence the quality of intake and the choice of food was very constant. Thus, pregnant women with low caloric consumption run the risk of getting an inadequate supply of certain nutrients.

The mean weight increase was, in our series, equal to that usually found in normal pregnancy (Lunell 1966). During normal pregnancy there is a considerable storage of fat by the mother (Taggart *et al.* 1967). Whether this can be or ought to be influenced by dietary changes can not be answered in this study.

The results indicate that dietary instructions to pregnant women with special disorders such as diabetes mellitus, must be done with great care in order to influence deep-rooted patterns in their choice of food.

## SUMMARY

The dietary pattern of normal pregnant women was analysed using the 24-hour recall method. Fifteen women were followed from the first to the third trimester and fifty-eight were studied

once during pregnancy. In general the mean consumption of calories and nutrients was similar to that of the general population with a high intake of fat and a relatively low iron intake.

### *Acknowledgement*

This study has been performed with financial support mainly from Semper Fond for Näringsforskning. The data from the interviews were analysed with a computer at Statens Institut för Folkhälsoen. Nurse Ulla Klingvall is thanked for all her help.

### REFERENCES

- Abramson E. Födoämnestabeller Svenska Bokförlaget, Stockholm, 1960  
Blix G. Acta Soc. Med. Upsalien. 70 117 1965  
Carlgren G and Cramér K. Läkartidningen, 62 4248 1965  
Engström L. and Sterky G. Läkartidningen 63 4922, 1956  
Hallberg, L. Obstetrik & Gynækologi 3 59 1966  
Lunell N-O. Acta Obstet Gynecol Scand. 45 Suppl. 4 1966  
Sterky G. Acta Paediat Scand Suppl 135 185 1962  
Stevens H A. and Ohlson M A. J Am Diet. Ass 50 290 1957  
Teggart N R. Holliday R. M. Billerwicz W Z. Hybben F E. and Thomson A M. Br J Nutr 21 439 1957  
WHO Expert Committee. WHO Techn Rep Ser No 202, 1965

Received on April 5 1968

## THE SIGNIFICANCE OF ABSENCE OF ONE UMBILICAL ARTERY

BY

K. KRISTOFFERSEN

An abnormal number of vessels in the umbilical cord almost always means the absence of one of the umbilical arteries, aplasia arteriae umbilicalis (a.a.u.) This anomaly seems to have been mentioned for the first time by Falloppio (1523-1562) and the subject is described in details by Hyrtl (1870) who at autopsies, found some cases of a.a.u. in normal children and in malformed fetuses too.

Until the report by Benirschke and Brown (1955) a.a.u. was looked upon as an anatomical curiosity. These authors analysed 55 cases of a.a.u. and found that 27 had congenital malformations and they also noted a correlation between this anomaly and both toxæmia of pregnancy and various abnormalities of the placenta. During the last 12 years, some workers have been studying the frequency of a.a.u. the proportion of cases which have malformations, the connection with maternal diseases the pathogenesis and the clinical significance of a.a.u.

Little (1968) proposed that the finding of a.a.u. at delivery might be a useful sign leading to the possibility of immediate diagnosis of a treatable malformation of the newborn child.

The object of the present investigation is to review the literature and to analyse 41 of our own cases of a.a.u. and from this to state our present knowledge about the significance of a.a.u.

### *Review of the Literature*

Reports of the frequency of a.a.u. and the proportion of malformations combined with a.a.u. vary considerably probably

Table 1 Frequency of Aplasia Arteriae Umbilicalis and Proportions of Malformed Foetuses  
Reports from the Literature

Authors	Material	Number Examined	Aplasia Art. Umbilicalis		Proportion of Malformed Foetuses	
			Number	Per Cent	Number	Per Cent
Adler Lewenthal and Ben Adereth 1963	consecutive obstetrical	2000	19	0.95	4	72.2
Adrian 1966	consecutive obstetrical	3688	25	0.68	5	20
Angiolillo and Picinelli 1966	consecutive obstetrical	1000	2	0.20	2	100
Bednarszke and Bourne 1960	consecutive obstetrical	1,000	15	1.00	7	46.6
Calmus and M'Kee 1964	consecutive obstetrical	2000	20	1.00	2	10
Carlier Mattern and Jean 1966	consecutive obstetrical	4138	33	0.79	5	15.2
Froelich and Fujikura 1966	consecutive single births	26339	203	0.76	58	28.6
	of these: white	11371	139	1.22	32	23
	of these: negroes	13058	57	0.44	24	42.1
	of these: others	2110	7	0.33	3	28.6
Fujikura 1964	consecutive obstetrical	5972	58	0.61	7	18.4
	of these, mic. ocopy of cord	1744	15	0.80		





Table 1 Frequency of *Aplasia Arteriae Umbilicalis* and Proportions of *Malformed Foetuses* Reports from the Literature

Authors	Material	Number Examined	Aplasia Art. Umbilicalis		Proportion of Malformed Foetuses	
			Number	Per Cent	Number	Per Cent
Adler Lewenthal and Ben Adereth 1963	consecutive obstetrical	2000	19	0.95	4	22.2
Adrian 1966	consecutive obstetrical	3688	25	0.68	5	20
Angiullo and Piccinelli 1966	consecutive obstetrical	1000	2	0.20	2	100
Dentrickie and Bourne 1960	consecutive obstetrical	1560	15	1.00	7	46.6
Cauna and McInee 1964	consecutive obstetrical	2000	20	1.00	2	10
Garner Matteau and Jean 1966	consecutive obstetrical	4148	33	0.79	5	15.2
Freulich and Fujikura 1966	consecutive single births	26539	203	0.76	58	28.6
Fujikura 1964	of these: white	11371	139	1.22	32	23
	of these: negroes	13058	57	0.44	24	42.1
	of these: others	2110	7	0.33	2	28.6
	consecutive obstetrical	5972	8	0.61	7	18.4
	of these: ml. of cord in specimen	1744 of 274	15	0.80	4	26.7

(1965) and *Peckham and Yerushalmy* (1965) did not find any cords with a.a.u. in 108 and 66 pairs of twins, respectively. In abortions *Javert* (1957) and *Thomas* (1961) find 2.4 and 2.7 per cent with a.a.u.

Among 41 pairs of twins referred to in the literature a.a.u. was found in one twin out of 39 pairs and in both of 2 pairs of dizygotic twins. Of these 41 pairs of twins 4 were monoamniotic, 14 monochorionic 19 dichorionic, of which 7 were of the same sex and in 4 cases the placentae were not described. In other words of 41 pairs of twins a minimum of 18 were monozygotic, all with discordant occurrence of a.a.u. and a minimum of 12 pairs were dizygotic twins with concordant occurrence of a.a.u. in only 2 cases. In 11 pairs of twins the zygosity could not be determined by means of the available information.

In 19 pairs of twins with discordant a.a.u. the birthweights were known. The smaller twin was the one with a.a.u. in 16 cases and the bigger twin in 1 case (*Benirschke and Brown* 1955 *Thomas* 1961 *Seki and Strauss* 1964) while in 2 cases of monoamniotic twins the birthweights were equal (*Moesstrup* 1969 *Wharton et al.* 1968). These 4 monoamniotic twins had no malformations.

*German et al* (1962) *Lenoski and Medow* (1962) *Levis* (1962) *Uchida Bowman and Wang* (1962) *Henrichs and Allen* (1963) and *Seki and Strauss* (1964) have together found 25 patients with autosomal trisomy of whom 14 also had a.a.u. *Kajdi et al* (1963) found a.a.u. in two out of four thalidomide malformed babies, and *Dunn Fisher and Kohler* (1962) reported a.a.u. in one of two dizygotic twins both with severe malformations caused by thalidomide. *Molz* (1965) mentioned that most of the children with a.a.u. and skeletal malformations in his autopsy material date from the period when thalidomide was in use in Western Germany but there are not any prospective studies to prove a causal relationship between a.a.u. and the use of thalidomide.

The ratio between males and females with a.a.u. is 1/1 (*Benirschke and Brown* 1955 *Froelich and Fujikura* 1966). Three authors (*Hyrtl* 1870 *Seki and Strauss* 1964 and *Molz* 1965) find a preponderance of boys among the children with a.a.u.

because different methods have been used for collecting cases and for examining the cords. But even if a consecutive collection of cords from one obstetrical department is studied, it is impossible to rule out some selection because some departments admit all deliveries while others have a relatively high proportion of complicated cases.

Table I shows the reported findings over the last 12 years with a description of the method of collecting cords, the number and incidence of a.a.u. and the proportion of malformations. The incidence of a.a.u. in reports from obstetrical departments varies from 0.2 to 1.22 per cent, and the corresponding results from pathological departments, based on autopsies, from 2.7 to 12 per cent. In very rare cases it is stated that the two umbilical arteries join one another near the placenta, (Hyrtl 1870 Little 1961 Seki and Strauss 1964) and fusion of the two umbilical arteries to form a single trunk within the foetal abdomen, has also been seen at autopsy (Seki and Strauss 1964). However, the most common abnormal finding is that one of the umbilical arteries, left or right with equal frequency, is absent from its origin. (Bourne and Benirschke 1960 Faerman 1960 Bridges and Morton 1964 Molz 1965) and the iliac and femoral arteries on the same side may be hypoplastic (Kajii Shimohara Akichuki Dohmen and Akichika 1963).

The proportion of malformations associated with a.a.u. varies from 0 to 100 per cent, but in the largest series the figure ranges from 15 to 48 per cent, and the proportion is even greater (from 49.1 to 93 per cent) in autopsy material.

The malformations may occur in all parts of the body. However, some authors find a preponderance in some systems, for example, the gastrointestinal and skeletal systems (Fujikura 1964 Molz 1965), the cardiovascular system (Seki and Strauss 1964), the central nervous system and the heart (Benirschke and Brown 1955) and the skeletal system alone (Froelich and Fujikura 1966). Almost all authors find that in most cases there are multiple malformations in different organs in the same child.

Some authors find that the incidence of a.a.u. in twins is greater than normal, namely 3.5 to 4 per cent (Benirschke and Bourne 1960 Thomas 1961) while Papadatos and Paschos

(1965) and *Pockham and Yerushalmy* (1965) did not find any cords with a.a.u. in 108 and 66 pairs of twins, respectively. In abortions *Japert* (1957) and *Thomas* (1961) find 2.4 and 2.7 per cent with a.a.u.

Among 41 pairs of twins referred to in the literature a.a.u. was found in one twin out of 39 pairs and in both of 2 pairs of dizygotic twins. Of these 41 pairs of twins 4 were monoamniotic, 14 monochorionic, 19 dichorionic, of which 7 were of the same sex, and in 4 cases the placentae were not described. In other words of 41 pairs of twins a minimum of 18 were monozygotic all with discordant occurrence of a.a.u., and a minimum of 12 pairs were dizygotic twins with concordant occurrence of a.a.u. in only 2 cases. In 11 pairs of twins the zygosity could not be determined by means of the available information.

In 19 pairs of twins with discordant a.a.u. the birthweights were known. The smaller twin was the one with a.a.u. in 16 cases and the bigger twin in 1 case (*Bernschke and Brown* 1955 *Thomas* 1961 *Seki and Strauss* 1964) while in 2 cases of monoamniotic twins the birthweights were equal (*Mønstруп* 1969 *Wharton et al.* 1968). These 4 monoamniotic twins had no malformations.

*German et al.* (1962) *Lenoski and Medovy* (1962) *Lewis* (1962) *Uchida Bowman and Wang* (1962) *Henrichs and Allen* (1963) and *Seki and Strauss* (1964) have together found 25 patients with autosomal trisomy of whom 14 also had a.a.u. *Kajli et al.* (1963) found a.a.u. in two out of four thalidomide malformed babies and *Dunn, Fisher and Kohler* (1962) reported a.a.u. in one of two dizygotic twins both with severe malformations caused by thalidomide. *Molz* (1965) mentioned that most of the children with a.a.u. and skeletal malformations in his autopsy material date from the period when thalidomide was in use in Western Germany but there are not any prospective studies to prove a causal relationship between a.a.u. and the use of thalidomide.

The ratio between males and females with a.a.u. is 1/1 (*Bernschke and Brown* 1955 *Froelich and Fujikura* 1966). Three authors (*Hyrtl* 1870 *Seki and Strauss* 1964 and *Molz* 1965) find a preponderance of boys among the children with a.a.u.,

but this was in selected autopsy material in which there are usually more males.

Some authors have noticed that children with a.a.u. have lower birthweights than normal (*Benirschke and Brown 1955* *Adler et al 1963* *Carrier et al 1966*) However *Seki and Strauss (1964)* find their birthweights as expected for the gestational age when allowance is made for the smaller weights of children with multiple malformations.

*Froelich and Fujikura (1966)* found the incidence of a.a.u. greater in white people (1.22 per cent) than in negroes (0.44 per cent) in a prospective study collected from 12 clinics, some treating mostly white patients and others mostly negroes, and including a total of 11,371 and 13,058 mothers respectively. Virtually the same figures were reported by *Peckham and Yerushalmy (1965)* 1.11 per cent a.a.u. on examination of 3597 white babies and 0.43 per cent of 1,167 negroes.

The perinatal mortality is high in cases of a.a.u. (13-30 per cent) primarily because of the many lethal malformations, but there is also an increased mortality among children without detectable anomalies. (*Little 1961* *Fujikura 1964* *Froelich and Fujikura 1966* *Peckham and Yerushalmy 1965*)

As to the age of the mothers of the children with a.a.u. *Benirschke and Brown (1955)* found that 21 of 44 (48 per cent) were more than 30 years and *Froelich and Fujikura (1966)* find a slight preponderance of mothers over 20 years of age. Other authors however could not find any difference from the normal age distribution (*Little 1961* *Cairns and McLes 1964* *Seki and Strauss 1964*) *Little (1961)* found a slight preponderance of multiparae but others report normal distribution with regard to parity.

A significant preponderance of diabetes mellitus is found among mothers of children with a.a.u. *Seki and Strauss (1964)* had an incidence of 14 per cent (7/50) and *Froelich and Fujikura (1966)* 6.4 per cent (13/203). A slightly greater occurrence than expected but not significant, is found in mothers suffering from neuro-psychiatric diseases (*Fujikura 1964* *Froelich and Fujikura 1966*)

*Benirschke and Brown (1955)* found that 30 per cent of the

mothers had preeclampsia and polyhydramnios occurred 4 times more than expected in their series. They also found that 18 of 46 placentae (39 per cent) appeared to have anomalies. These were mostly circumvallate placentae, velamentous insertions of the cord and infarcts. Seldi and Strauss (1964) found that 6 of their 52 cases had a velamentous insertion of the cord and 7 of 52 placentae weighed less than expected in relation to the weight of the child, and the same correlation was found in the large series published by Froelich and Fujikura.

Seldi and Strauss (1964) found that 15 out of 50 mothers of children with a.a.u. had a history of one or more abortions and Adrian (1966) reported that, out of 25 such mothers, 17 had a history of 33 pregnancies only 16 of which resulted in a mature living child.

In the consecutive obstetrical series four different methods of demonstrating the vessels in the cord have been used

1) Inspection of the cut-end of the fresh umbilical cord at birth. This method has been most frequently used, and has yielded an incidence of a.a.u. ranging from 0.2 to 0.8 per cent.

2) Naked eye inspection of a slice of the umbilical cord after fixation by formalin or fixative solution (Cairns and McKee 1964 Peckham and Yerushalmy 1965)

3) Microscopic examination of all cords (Bentrschke and Bourne 1960 Lyon 1969 Froelich and Fujikura 1966) By these two methods the incidence of a.a.u. is found to be somewhat higher (0.76-1.1 per cent)

4) Inspection of the umbilical cord after fixation in glacial acetic acid (Krapitz 1967) This author only examined 265 cords by this method and found 3 with a.a.u. (1.13 per cent) but he claims that with this treatment the cord becomes translucent and rubbery in consistency so that the vessels are more easily seen.

#### *Own Material and Methods*

A study has been made of 41 children with a.a.u. Of these, 16 have previously been referred in a lecture in Dansk Selskab for Obstetrik og Gynækologi on the 24th of January 1964. These

are collected in a two year period from The Maternity Department B Rigshospitalet, Copenhagen (17 cases) The Department of Obstetrics and Gynaecology D Odense County and City Hospital, Odense (16 cases) and The Municipal Maternity Clinic in Odense (8 cases)

Two different methods have been used for the examination of the cords

1) From 9/1 1964 to 23/12 1964 1129 cords were fixed in formalin and examined later by the author. In this way 13 cases (1.15 per cent) of a.a.u. were demonstrated.

2) The remaining 28 cases of a.a.u. were found by a routine inspection of fresh cords carried out by the staff of the various departments. 7622 children were born during the period of the examination. This gives an incidence of 0.37 per cent with a.a.u. The difference between the results obtained by the two methods is significant ( $p < 0.01$ ). Twenty-eight of the 41 cords with a.a.u. were examined microscopically while the remaining 13 were only inspected macroscopically.

In the whole series there were 106 pairs of twins and the cords from 26 of these were examined after fixation in formalin.

### Results

Table II a, b and c show how the mothers of the 41 children are distributed in relation to age, gravidity and parity. 11 of the 41 (26.8 per cent) were more than 30 years of age and 7 (17.1 per cent) had a history of 4 or more pregnancies. This distribution does not differ from that found in a study of all mothers delivered in Odense County and City Hospital during the period of the examination.

Prior to the pregnancy studied the 41 mothers had a history of 58 pregnancies with results as shown in Table III. It is seen that their obstetrical histories are loaded with 13 spontaneous abortions (22.4 per cent) and a total foetal loss of 31 per cent, because 5 children died within 3 weeks of birth. The 5 legal abortions have been excluded. The 18 unsuccessful pregnancies were shared by 14 mothers while 27 mothers had not lost any children.



Table II a. Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicalis by Age

Age years	≤20	>20≤25	>25≤30	>30≤35	>35≤40	>40	total
Number	5	16	9	11	1	2	41

Table II b. Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicalis by Gravida

Gravida	I	II	III	>IV	total
Number	16	8	10	7	41

Table II c. Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicalis by Parity

Parity	I	II	III	>IV	total
Number	21	9	7	5	41

Table III Results of the 58 Pregnancies Prior to 41 Pregnancies Which Resulted in Children with Aplasia Arteriae Umbilicalis

	Spontaneous abortions	Legal abortions	Perinatal deaths	Living children	Total
Number	13	5	5	35	58
Per cent	22.4	8.6	8.6	60.4	100

Eight of the mothers had 11 diseases and 14 mothers had 17 complications of their pregnancies as shown in Tables IV a and b. The four cases of insulin treated diabetes mellitus are 4 times more than expected. Among anomalies of the placenta infarcts were found in 8 cases and velamentous insertion of the umbilical cord in 2 cases. Three of the mothers had a family history of congenital malformations. In one family four girls had congenital dislocation of the hip in another family one child had congenital heart disease and one had died when 8 months old from an unknown cause. In a third family the mother had previously given birth to a hydrocephalic child (no. 6 in Table VI).

One of the 41 children, a second twin (935 g) was a stillborn

are collected in a two year period from The Maternity Department II Rigshospitalet Copenhagen (17 cases) The Department of Obstetrics and Gynaecology D Odense County and City Hospital Odense (16 cases) and The Municipal Maternity Clinic in Odense (8 cases)

Two different methods have been used for the examination of the cords

1) From 9/1 1964 to 23/12 1964 1129 cords were fixed in formalin and examined later by the author. In this way 13 cases (1.15 per cent) of a.a.u. were demonstrated.

2) The remaining 28 cases of a.a.u. were found by a routine inspection of fresh cords carried out by the staff of the various departments. 7622 children were born during the period of the examination. This gives an incidence of 0.37 per cent with a.a.u. The difference between the results obtained by the two methods is significant ( $p < 0.01$ ). Twenty-eight of the 41 cords with a.a.u. were examined microscopically while the remaining 13 were only inspected macroscopically.

In the whole series there were 106 pairs of twins and the cords from 26 of these were examined after fixation in formalin.

### Results

Table II a b and c show how the mothers of the 41 children are distributed in relation to age, gravidity and parity. 11 of the 41 (26.8 per cent) were more than 30 years of age and 7 (17.1 per cent) had a history of 4 or more pregnancies. This distribution does not differ from that found in a study of all mothers delivered in Odense County and City Hospital during the period of the examination.

Prior to the pregnancy studied the 41 mothers had a history of 58 pregnancies with results as shown in Table III. It is seen that their obstetrical histories are loaded with 13 spontaneous abortions (22.4 per cent) and a total foetal loss of 31 per cent because 5 children died within 3 weeks of birth. The 5 legal abortions have been excluded. The 18 unsuccessful pregnancies were shared by 14 mothers while 27 mothers had not lost any children.

Table II Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicales by Age

Age: years	≤20	>20 ≤25	>25 ≤30	>30 ≤35	>35 ≤40	>40	total
Number	5	16	9	8	1	2	41

Table II b Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicales by Grandity

Pregnancy	I	II	III	>IV	total
Number	16	8	10	7	41

Table II Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicales by Parity

Parity	I	II	III	>IV	total
Number	21	8	7	5	41

Table III Results of the 58 Pregnancies Prior to 41 Pregnancies Which Resulted in Children with Aplasia Arteriae Umbilicales

	Spontaneous abortions	Legal abortions	Perinatal deaths	Living children	Total
Number	13	5	5	35	58
Per cent	22.4	8.6	8.6	60.4	100

Eight of the mothers had 11 diseases and 14 mothers had 17 complications of their pregnancies as shown in Tables IV a and b. The four cases of insulin treated diabetes mellitus are 4 times more than expected. Among anomalies of the placenta infarcts were found in 8 cases and velamentous insertion of the umbilical cord in 2 cases. Three of the mothers had a family history of congenital malformations. In one family four girls had congenital dislocation of the hip. In another family one child had congenital heart disease, and one had died when 11 months old from an unknown cause. In a third family the mother had previously given birth to a hydrocephalic child (no. 11 in Table VI).

One of the 41 children, a second twin (935 g) was a stillborn

Table IV *Mothers Diseases Complications in Pregnancy and Anomalies of Placenta in 41 Cases of Aplasia Arteriae Umbilicalis*

Eight Mothers with Following Diseases.		Fourteen Mothers with Following Complications in Pregnancy:	
Diabetes mellitus	4	Preeclampsia	
Epilepsy	2	Essential Hypert.	
Mitral stenosis	1	Proteinuria	
Pulmonary TB	1	Oedema	
Psycho-neurosis	1	Threatened abortion	
Uterine fibroids	1	Bleeding of unknown cause	
Jaundice of pregnancy	1	Anaemia	
a		Rhesus iso-immunization	
		ABO-iso-immunization	
		Hydramnios	
Anomalies of Placenta.		b	
Infarcts	8		
Velamentous insertion of the cord	2		
Placenta marginata	1		
Placenta praevia	2		
Vasa praevia	1		
8-shaped placenta	1		
Retained placenta	1		
c			

monster whose sex could not be determined otherwise there were 20 males and 20 females

The birthweights of the children are distributed as shown in Table V Two were immature ( $\leq 1000$  g) 14 premature and 24 weighed more than 2500 g Of the 29 children born of mothers without diabetes mellitus and in whom the gestational age was known, 9 had a birthweight higher and 20 lower than that calculated from the formula of Streeter The average weight of the 29 children was 288 g less than expected The fate of the 41 children is also analysed in Table V 5 were stillborn, 6 died within 3 weeks so the mortality was 26.8 per cent. Eleven (26.8 per cent) had congenital malformations The malformations are described briefly in Table VI together with the mother's age parity associated diseases and complications It is remarkable

Table V Distribution in Weight-Classes and Its Correlation to the Fate of 41 Children with *Aplasia Arteriae Umbilicalis*

Weight in Grams	$\leq 1000$	$> 1000 \leq 2500$	$> 2500$	Unknown	Total
Total number	2	14	24	1	41
Living	0	6	24	0	30
Dead	0	5	0	1	6
Stillborn	2	3	0	0	5
Malformations	1	8	1	1	11

that only one of the 24 mature children had malformations (no. 4) while 8 of 14 premature and 1 of 2 immature babies were malformed. Only 2 of the 11 malformed babies are alive (no. 4 and 11). Seven had multiple anomalies involving various systems, and 5 of these were severe enough to be incompatible with life. No malformations were found in the 2 stillborn babies but only one of them had autopsy.

There was only one second twin with a.a.u. among 106 pairs of twins (no. 3 in Table VI).

In two cases (no. 1 and 2) the malformation was diagnosed shortly after birth because of the finding of a.a.u. which led to a thorough examination of the babies, including the passage of an oesophageal catheter, a chest X ray and an E.C.G.

The 2261 cords from Odense County and City Hospital were examined routinely both at the placenta and at the umbilicus and in no case was there any discrepancy between the two observations.

The incidence of a.a.u. in relation to the seasons of the year is mentioned by Peckham and Yerushalmy (1965) who found that the last menstrual period of the mothers giving birth to children with a.a.u. was in the quarter July-September in 24 out of 51 mothers which was more than twice as often as in each of the other three quarters of the year. In the present investigation the time of conception could be determined in 39 mothers and the distribution in the four quarters of the year was 8-12-12 and 7 respectively so that 24 of the 39 fell in the half year April-September.

Table IV *Mothers Diseases Complications in Pregnancy and Anomalies of Placenta in 41 Cases of Aplasia Arteriae Umbilicalis*

Eight Mothers with Following Diseases:		Fourteen Mothers with Following Complications in Pregnancies:	
Diabetes mellitus	4	Preeclampsia	
Epilepsy	2	Essential Hypert.	
Mitral stenosis	1	Proteinuria	
Pulmonary TB	1	Oedema	
Psycho-neurosis	1	Threatened abortion	
Uterine fibroids	1	Bleeding of unknown cause	
Jaundice of pregnancy	1	Anaemia	
a		Rhesus iso-immunization	
		ABO-iso-immunization	
		Hydramnios	
Anomalies of Placenta.		b	
Infarcts	8		
Velamentous insertion of the cord	2		
Placenta marginata	1		
Placenta praevia	2		
Vasa praevia	1		
8-shaped placenta	1		
Retained placenta	1		
c			

monster whose sex could not be determined, otherwise there were 20 males and 20 females

The birthweights of the children are distributed as shown in Table V. Two were immature ( $\leq 1000$  g), 14 premature and 24 weighed more than 2500 g. Of the 29 children born of mothers without diabetes mellitus and in whom the gestational age was known, 9 had a birthweight higher and 20 lower than that calculated from the formula of Streeter. The average weight of the 29 children was 288 g less than expected. The fate of the 41 children is also analysed in Table V. 5 were stillborn, 11 died within 3 weeks so the mortality was 26.8 per cent. Eleven (26.8 per cent) had congenital malformations. The malformations are described briefly in Table VI together with the mother's age, parity, associated diseases and complications. It is remarkable

Umbilical: Gestational Age Weight Length Sex and Their Mothers Age Gravidity Complications in Pregnancy

Weight Grams	Length cm	Sex	Age	Gravidity	Mothers	
					Parity	Diseases and Complications in Pregnancy
1580	44	F	23	I	I	Haemorrhage in pregnancy pre-eclampsia, velamentous insertion of the cord, placental infarct
2400	49	M	22	II	II	Placental infarct
935	22	F	19	III	II	Light-shaped placenta
3350	53	F	25	II	II	Diabetes mellitus and epilepsy
1180	34	M	27	III	II	Diabetes mellitus pre-eclampsia, hydramnios
1330		F	26	III	III	Well, but has previously given birth to child with hydrocephalus and spina bifida
2400	45	M	20	I	I	Psycho-neurosis treated by different drugs during pregnancy
2000	44	F	25	II	I	Threatened abortion in pregnancy
2250	49	F	25	I	I	0
		M	38	IV	IV	Retained placenta
1900	46	M	35	V	III	0

Table VI. *Records of Observed Malformations in 11 Children with Aplasia Arteriae Parity Diseases and*

No	J	No	Description of Malformations and Fate of Child	Gestational Age in Days
1	II	377/62	Oesophageal atresia operated died 11 days old	287
2	II	1784/62	Endomyocardial fibroelastosis coarctation of the aorta aplasia arteriae umbilicalis intraabdominalis died 3 days old	252
3	II	2078/62	Stillborn monster second twin, 3 limbs and small excrescences instead of head cystic kidney	180
4	II	2279/62	Single harelip alive 4 years old, well	259
5	B	1729/63	Stillborn anencephalic cervical rachischisis splenomegaly hypoplasia of the suprarenal glands	246
6	B	2084/63	Stillborn anencephalic club-feet hypoplasia of the suprarenal glands (1 g)	189
7	A	854/63-64	Housden's syndrome: atresia mm. abdominis malrotation of gut hypoplastic kidneys distended bladder vesico-umbilical fistula, club-feet died 20 days old	263
	II	A 220/6.-64	Tricuspid stenosis hypertelorism syndactyly died 2 days old	245
9	A	480/63-64	Hydrocephalus anophthalmia died soon after birth	270
10	D	222/64-65	Stillborn with omphalocele club-feet harelip	206
11	D	1924/64-65	Branchial fistula alive Two years old he was examined in paediatric department Diagnosis: branchial fistula nasal catarrh nummular eczema enlarged tonsils and adenoids and perhaps mental retarded	235



Table: Gestational Age, Weight, Length, Sex and Their Mothers' Age, Gravidity, Parity, Diseases and Complications in Pregnancy

Weight gms	Length cm	Sex	Mothers			
			Age	Gravidity	Parity	Diseases and Complications in Pregnancy
500	44	F	23	I	I	Haemorrhage in pregnancy, pre-eclampsia, velamentous insertion of the cord, placental infarct
400	49	M	22	II	II	Placental infarct
925	22	?	19	III	II	Eight-shaped placenta
3350	53	F	25	II	II	Diabetes mellitus and epilepsy
1100	34	M	27	III	II	Diabetes mellitus, pre-eclampsia, hydramnios
1330	?	F	28	III	III	Well, but has previously given birth to child with hydrocephalus and spina bifida
2400	45	M	20	I	I	Psycho-neurosis treated by different drugs during pregnancy
2000	44	F	25	II	I	Threatened abortion in pregnancy
2250	49	F	25	I	I	0
		M	28	IV	IV	Retained placenta
1900	46	M	25	V	III	0

*Follow up studies*

Questionnaires were answered by the mothers of 24 of the 30 children, who were alive on leaving hospital. They were asked about the children's general health and whether they had suffered from any diseases up to the age of 2-5 years. 15 children had been quite healthy since leaving hospital and were thriving.

Of the other 9 children, 4 had had many upper respiratory tract infections, one child was very thin and would not eat, one child had a history of otitis and fever, convulsions, was slender and would not eat, one was treated in the radiological department for a haemangioma (1.8×1.2 cm) on the abdominal skin just below the right costal margin, and one had been taken into the hospital for an inguinal hernia, but the hernia disappeared spontaneously. The last child was taken into the paediatric department for examination as a result of the answer on the questionnaire and the diagnoses are listed in Table VI (no 11).

From these questionnaires it is not possible to reach a firm conclusion whether these children's health and growth are any worse than normal. As quite a lot of them (6) are said to have recurrent infections, there are good reasons for paediatric follow up throughout childhood.

*Discussion and Conclusions*

The incidence of a.a.u. has been found to be significantly higher on macroscopic or microscopic examination after fixation than by routine inspection of fresh cords at birth. This is confirmed in the present investigation where 1.15 per cent and 0.37 per cent a.a.u. respectively were found by these two methods. It is concluded that the best way to pick up all cases of a.a.u. is to examine all cords macroscopically or microscopically after fixation in formalin or glacial acetic acid. In the present series fixation in formalin has made the vessels stand out clearly. However, comparative studies in the same umbilical cord (Fig 1) suggest that the glacial acetic acid method is even better, giving a translucent cord with the course of the vessels very clearly seen.

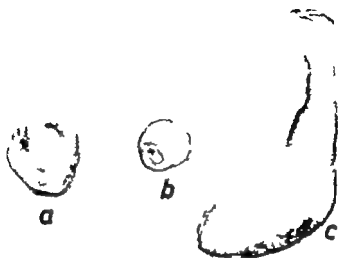


Fig. 1 Umbilical cord treated with (a) formalin-alcohol (b) and (c) treated with glacial acetic acid.

By these methods the incidence of a.a.u. has been found to range from 0.76 to 1.15 per cent. Although the patients in most obstetrical departments are somewhat selected to include more abnormal pregnancies than in the general population, it seems reasonable to estimate that the incidence of a.a.u. is between 0.5 and 1.0 per cent. The proportion of associated malformations is 25 to 50 per cent. The incidence of congenital malformations in the general population is about one per cent, therefore between 1/4 and 1/2 of children with severe malformations lack one umbilical artery.

Abnormalities of the placenta especially velamentous insertion of the cord and infarcts are found in association with a.a.u. more frequently than is expected. Maternal diabetes mellitus is found 4 times more often than expected in the present series, and this agrees with previously published results. There are more mothers than normal with illnesses although no single

disease is significantly increased, apart from diabetes mellitus. The incidence of preeclampsia and haemorrhage in pregnancy is a little more than normal

The present series includes 106 pairs of twins and only one child with a.a.u. was found. This agrees with the findings of *Papadatos* and *Paschos* (1965) and those of *Peckham* and *Yerushalmy* (1965) while the increased incidence of a.a.u. among twins referred to by *Benirschke* and *Bourne* (1960) and *Thomas* (1961) could not be confirmed. It is not possible to explain this discrepancy

As to the cause of a.a.u. and the many associated malformations *Benirschke* and *Brown* (1955) have proposed the following theories: a) a.a.u. might be one of a number of malformations in the same child and with the same cause b) it is also possible that the absence of one artery might cause an increased resistance to the umbilical blood flow with possible foetal hypoxia and consequently multiple malformations. They emphasise that the causative factor must be present for long periods of embryonic development in order to explain so many varied malformations and the fact that there is no familial occurrence of a.a.u. excludes a genetic cause

The discordance in monozygotic twins and the seasonal variations in the occurrence of a.a.u. indicate that environmental factors are more important. On the other hand the high incidence of miscarriages in the obstetrical history of the mothers bearing children with a.a.u. might indicate a hereditary disposition to congenital malformations

Whether a.a.u. is the cause of the associated malformations or a part of these with a separate common cause the existence of a.a.u. must indicate some exogenous factor. By exogenous is meant a factor outside the foetus and its placenta and might for instance be abnormal metabolism in the mother or drugs taken by her. In the present series congenital malformations were found in 9 out of 16 children with a.a.u. and a birthweight  $\leq 2500$  g, but only one out of 24 with a birthweight  $> 2500$  g had a minor malformation (harelip). These findings agree very well with those of *Seki* and *Strauss* who state that the birthweights of children with a.a.u. were as expected for the gesta-

tional age except for those with multiple malformations. So it may be concluded that children with both a.a.u. and low birth weight have a greater incidence of malformations than children with a.a.u. and normal birthweight. Among the twins recorded in the literature it was found that the twin with a.a.u. had a lower birthweight than the other one. These facts might indicate that a.a.u. could cause impaired nourishment of the foetus with resulting low birthweight and when this impairment is marked, it might also be the cause of multiple malformations.

*Rahbek Sørensen* (1953) found in his investigation of hypospadias in twins, that in 5 cases where this anomaly was discordantly present, the twin with hypospadias had a lower birthweight than the other twin. No examination of the cord vessels was done in his series, but it is interesting to find this similarity to the findings in a.a.u. because it supports the theory that an impaired supply of oxygen and/or nutrition to the foetus may be accompanied by or perhaps be the cause of malformations.

The clinical significance of the finding of a.a.u. as a means of diagnosing a congenital malformation is much reduced by the fact that the malformations are most often severe and multiple. However in a few cases, (twice in the present series) the finding turns out to be of practical clinical importance.

In experimental teratology in animals and in prospective studies of human malformations the demonstration of a.a.u. is supposed to be of significance as an indicator of exogenous causative factors. However the problem is very complicated because we must allow as emphasized by *Bro-Rasmussen and Hansen* (1961) for the possibility that exogenous factors may work on a background of individual gene-patterns which govern a greater or lesser disposition to malformations.

Follow-up of children with a.a.u. has been done by a few authors. *Benirschke and Bourns* (1960) on examination of 10 children at the age of 6 months found no special disorders, and *Froelich and Fujikura* (1966) examined 99 children at the age of 12 months. The latter authors point out that there were no uro-genital disorders except for three cases of hypospadias. *Fujikura* (1964) found that three out of 16 affected children were retarded in their movement at 12 months. *Little* (1961) mentions

that 8 out of 15 living children had physical findings compatible with congenital abnormalities two of these showing congenital heart disease. The results in the present follow up study which showed that 6 out of 24 children were susceptible to infections, suggest that there is good reason in the future to follow up these children in paediatric clinics and observe their motor and mental development. It might be of particular interest to examine the legs to see if they are equally developed remembering that unilateral hypoplasia of iliac and femoral arteries may be found in these children.

### SUMMARY

A review of the literature of the last 12 years on aplasia arteriae umbilicalis (a.a.u.) is presented. Together with 41 personal cases the reports are analysed and the following conclusions made

- 1 Aplasia arteriae umbilicalis occurs in 0.5 to 1 per cent of all cords but it is 2 to 3 times more frequent in aborted and premature foetuses than in mature babies. The incidence in girls and boys is the same

2. Congenital malformations are found in 25 to 50 per cent of children with a.a.u. They tend to be multiple and lethal and are far more common in premature babies

- 3 The incidence of a.a.u. in twins is found by some authors to be increased to about 4 per cent, but in other series including the present one no such increase is found. A.a.u. is perhaps a little more frequent in monozygotic than dizygotic twins but was not found to be concordant in any of 18 pairs of monozygotic twins. The twin with a.a.u. practically always has lower birthweight than the other one

- 4 In children of diabetic mothers the incidence of a.a.u. is increased 4 to 6 times. Medical diseases as a whole but perhaps especially neuro-psychiatric and cardiac disorders are found more commonly among mothers bearing children with a.a.u.

- 5 Complications of pregnancy such as preeclampsia, hypertension and ante-partum haemorrhage often due to placenta praevia, as well as other abnormalities of the placenta seem to be more frequent than normal.

6 Demonstration of the umbilical vessels is most reliable if a piece of all cords is fixed in formalin, or better in glacial acetic acid, for future examination, as the vessels are then clearly seen by the naked eye.

7 Follow-up of the development and health of the children born with a.a.u., and without other visible anomalies, has not given consistent results and therefore this problem needs further investigation.

8 The high incidence of congenital malformations in association with a.a.u. is supposed to indicate that these malformations are caused by exogenous factors in the environment of these children.

### Acknowledgement

I am grateful to overlæge P. Viatum for having supplied me with the material from the Municipal Maternity Clinic in Odense and for having personally examined the cords.

### REFERENCES

- Adler J, Lewenthal H and Ben-Adeneth N Harefuah 83 288 1953  
 Adnan K Obstetrik & Gynækologi 3 Akademi-förlaget Göteborg, 1956  
 204-209  
 Angiolillo M and Piccinelli M L Boll. Soc Ital Biol Sper 42 19 1966  
 Benirschke K and Bowser W H Obst. and Gynec. 6 399 1955  
 Benirschke K and Bowser G L Am J Obst. & Gyn 79 251 1960  
 Bowser G L and Benirschke K Arch Dis Childh. 35 534 1960  
 Bridges J B and Martow W R M Aust. Rec. 148 103 1954  
 Bro-Rasmussen F and Hansen O M Ugeskr Læg. 123 1 1961  
 Cairns J D and McKee J Canad M.A.J 91 1071 1954  
 Carrier C, Messieu P and Jean C Canad M.A.J 94 1001 1966  
 Dunn P M, Fisher A M and Kohler H G Am J Obst. & Gyn. 84  
 348 1952  
 Falloppio G cited by Gonder and Koller Gynækologia 157 177 1964  
 Fairman E Arch Dis Childh 35 285 1950  
 Froelich L A and Fujikura T Am J Obst. & Gyn. 94 274 1966  
 Fujikura T Am J Obst. & Gy 88 829 1954  
 German J L, Reublin J K, Harrison Ph. A, Donovan D J, Hogan W J  
 and Beern A J J Pediatr 60 503, 1962  
 Gömöri Z and Koller Th Gynækologia 157 177 1964  
 Heinrichs E H and Allen S W Clin. Pediatr 2 25 1963

- Hyrtl J Braumüller Vienna 1870 Die Blutgefäße der menschlichen Nach-  
geburt in normalen und abnormalen Verhältnissen
- Javert C T Spontaneous and Habitual Abortion New York, 1957
- Kajit T Shimohara M Kichuchi K. Dohmen S and Akichika M.  
Lancet II 889 1963
- Kravitz H Amer J Dis Child. 113 363 1957
- Lenoski E. F. and Medovy H Canad M.A.J 87 1229 1962
- Lewis A. J Lancet I 866 1962
- Little W A Obst. & Gyn. 17 695 1961
- Lyon F A. Obst. & Gyn 16 719 1950
- Moestrup J K. In press
- Molz G Helv Paediat. Acta 20 403 1965
- Papadatos C and Paschos A. Obstet. & Gynec 26 367 1965
- Peckham C H and Yerushalmy J Obstet. & Gynec. 26 359 1965
- Rahbek Sorensen H Hypospadias Thesis Copenhagen 1953
- Seki M and Strauss L Arch. Path 78 446 1954
- Streeter G L in Williams Obstetrics 11 Ed Appleton-Century-Crofts Inc.  
New York, 1956 p 185
- Thomas J Geburtsh und Frauenheilk. 21 984 1961
- Uchida I A. Bowman J M. and Wang, H C New Engl J Med. 266  
1198 1962
- Wharton B Edwards J H and Cameron A. H J Obst. Gyn. Brit  
Cwith. 75 158 1968

Received on April 5 1968



## THE INFLUENCE OF PROPHYLACTIC EXTERNAL CEPHALIC VERSION ON THE INCIDENCE OF BREECH DELIVERY

A Retrospective Study

BY

JOHANNES E. BOCK

It is well known that the delivery of a breech presentation involves a greater risk to the foetus than that of a cephalic presentation. A part of this increased risk is due to the fact that more premature babies are delivered as breech presentations, but even when these are excluded the risk is still greater. The perinatal mortality rate for infants weighing over 2,500 g is higher in breech presentations (Cannell and Dodge 1934 Fischer Rasmussen and Trolle 1967) and the incidence of mental and motor retardation is greater among children delivered in this way (Bishop Israel and Briscoe 1965).

In view of this increased risk to the foetus it would seem reasonable to carry out prophylactic external cephalic version in order to convert a breech presentation to the less dangerous vertex presentation before labour commences.

Many authors favour this procedure and consider that it results in a decrease in the incidence of breech deliveries (Jokela 1949 Dalley 1952 Kuhnelt 1954 v Friesen 1957 Belscharr and Townsend 1960). Von Friesen (1957) finds a 3.03 per cent incidence of breech delivery (among 4,448 single births with a birth weight of more than 2,500 g) when external version is not performed and a 1.70 per cent incidence (among 4,181 single births) when external version is performed. This decrease of 1.33 per cent is statistically significant.

Other authors have questioned the value of the prophylactic external cephalic version (Olow and Posse 1950 Brosser 1956 Morgan 1962 Geenhuil 1963). Brosser (1956) states that ex

ternal version can only be performed in cases where spontaneous version would have taken place and, therefore he finds external version of breech presentations of little value. *Morgan* (1962) is of the opinion that the foetal mortality rate cannot be reduced by substituting the head for the breech because the version will carry with it a certain foetal mortality. *Greenhill* (1965) finds only a slight difference in the mortality of vertex and breech presentations and, therefore he seldom performs external version.

The incidence of breech presentation at birth is usually given as 3-4 per cent. In the 32nd week 25 per cent of breech presentations have been found (*Vartan* 1945). Spontaneous version is responsible for the decrease. According to *Vartan* (1945) *Jokela* (1949) and *Kühnel* (1954) spontaneous version after the 34th week occurs so rarely that it may be assumed that the optimum time for external version is from the 34th week onwards.

External version without anaesthesia is preferable. In *Fell's* (1953) series foetal loss due to version was 2 per cent when the patients were anaesthetized, whereas there were no foetal deaths when the external versions were carried out without anaesthesia. When the patient is conscious only gentle manipulations will be tolerated and this is a safeguard against accidents which mostly result from the use of excessive force. The risks to the foetus are partial separation of the placenta, the onset of premature labour following rupture of the membranes and prolapse of the cord. There is virtually no risk to the mother. Cases of rupture of the uterus as a consequence of version have been reported but this happens very seldom and the presence of a uterine scar due to previous Caesarean section or myomectomy does not contraindicate the performance of external version (*Fell* 1953).

External version is contraindicated in patients with a history of antepartum haemorrhage, in patients with hypertension (*Fell* 1953) and of course in patients where elective Caesarean section is planned and in multiple pregnancies. According to *Greenhill* (1965) uterine scars are a contraindication too.

The incidence of successful prophylactic external version varies. *Brosset* (1956) found that only 30 per cent of the ver

alone performed were successful. This incidence is low compared with that reported by other authors (Jokela 1949 Fell 1953 Kühnel 1954 Friedlander 1966). The majority of these authors were successful in about 80 per cent or more of their cases. Successful version becomes less likely as term is approached, presumably for the same reasons that spontaneous version becomes less frequent. The increasing size of the foetus and the tendency to fixation of the presenting part are probably the main factors concerned. Apart from the gestation period the factors which make successful version less likely include extended legs, scanty liquor amnii, a thick abdominal wall, and an irritable uterus.

### *Material*

In order to get an impression of the influence of prophylactic external cephalic version on the incidence of breech presentation two 5-year periods have been analysed and compared.

During the first period January 1 1957–December 31 1961 there was not any special department of obstetrics in the Esbjerg area. No attempts at external version were made. Most deliveries took place in the patients' homes. 4,316 consecutive single births weighing more than 2,500 g were collected during this period.

During the second period April 1 1962–March 31 1967 prophylactic external version was performed at the Department of Obstetrics in the same area. 6,038 single births weighing more than 2,500 g took place in the Department during this period. Few deliveries in the area still took place in the patients' homes. Among these there were not any breech presentations weighing more than 2,500 g. The incidence of breech delivery in the area may therefore be somewhat lower than the incidence in the Department.

Attempts at version were performed without anaesthesia from the 34th week onward up to one day before term.

### *Results*

During the period 1957–1961 4,316 single births weighing more than 2,500 g were registered. The incidence of breech delivery was 3.15 per cent (Table I).

ternal version can only be performed in cases where spontaneous version would have taken place and, therefore he finds external version of breech presentations of little value *Morgan* (1962) is of the opinion that the foetal mortality rate cannot be reduced by substituting the head for the breech because the version will carry with it a certain foetal mortality *Greenhill* (1965) finds only a slight difference in the mortality of vertex and breech presentations and, therefore he seldom performs external version.

The incidence of breech presentation at birth is usually given as 3-4 per cent. In the 32nd week 25 per cent of breech presentations have been found (*Varran* 1945) Spontaneous version is responsible for the decrease According to *Varran* (1945) *Jokela* (1949) and *Küllinck* (1954) spontaneous version after the 34th week occurs so rarely that it may be assumed that the optimum time for external version is from the 34th week onwards.

External version without anaesthesia is preferable In *Fell's* (1953) series foetal loss due to version was 2 per cent when the patients were anaesthetized whereas there were no foetal deaths when the external versions were carried out without anaesthesia. When the patient is conscious only gentle manipulations will be tolerated and this is a safeguard against accidents which mostly result from the use of excessive force The risks to the foetus are partial separation of the placenta the onset of premature labour following rupture of the membranes and prolapse of the cord. There is virtually no risk to the mother Cases of rupture of the uterus as a consequence of version have been reported, but this happens very seldom and the presence of a uterine scar due to previous Caesarean section or myomectomy does not contraindicate the performance of external version (*Fell* 1953)

External version is contraindicated in patients with a history of antepartum haemorrhage in patients with hypertension (*Fell* 1953) and of course in patients where elective Caesarean section is planned, and in multiple pregnancies According to *Greenhill* (1965) uterine scars are a contraindication too

The incidence of successful prophylactic external version varies *Brosset* (1956) found that only 30 per cent of the ver

During the period 1962-1967 the incidence of breech delivery was 2.05 per cent (Table II)

The difference between the incidence of breech delivery when version was not performed and that when version was performed is 1.10 per cent. The decrease is statistically significant ( $p < 0.001$ ) The perinatal mortality rate for cephalic presentations over 2,500 g in the last 5-year period was 1.13 per cent. The perinatal mortality rate for the 124 deliveries of breech presentations was 4.91 per cent (Table III)

One of the six foetal deaths was due to a lethal malformation (anencephaly) The causes of the other foetal deaths were prolapse of the cord (2) Intracranial haemorrhage (1) and unknown (2) The corrected mortality rate is 4.04 per cent (Table III) For cephalic presentations the corrected mortality rate is 1.05 per cent.

340 attempts at external version were performed in 296 patients Two three or four attempts were made if version was not successful or if the presentation reverted after a successful version. The results are illustrated in Table IV

In the remaining 68 cases of breech deliveries attempts at version were not performed. 31 patients were admitted to the Department in labour without any antenatal care at the Department. 32 patients had a cephalic presentation at the antenatal clinic six to three weeks before term, but there may be a possibility of incorrect diagnosis since no radiographs were taken. Spontaneous version to a breech presentation is also a possibility In 5 cases external version was contraindicated. 2 patients had antepartum haemorrhage and in 3 patients elective Caesarean section was planned because of the breech presentation in association with previous classical Caesarean sections (2) and pelvic contraction (1) Previous lower segment Caesarean section was not considered as a contraindication to the performance of external version.

In 3 cases external version was followed by complications and one of these resulted in foetal death

IV granda 28 years old 4 weeks before the expected date of delivery the patient was found to have breech presentation. External version was performed 3 days after the version foetal movements disappeared, and labour

Table I. *Single Births over 2,500 g 1957-1961*

Total deliveries	4,316
Breech presentations	136
Incidence of breech delivery (per cent)	3.15

Table II. *Single Births over 2,500 g. 1952-1967*

Year	1952/63	1953/64	1954/65	1955/66	1956/67	Total
Total deliveries	848	1,105	1,199	1,373	1,533	6,058
Breech presentations	21	26	23	31	23	14
Incidence of breech delivery (per cent)	2.48	2.44	1.92	2.26	1.50	2.05

Table III. *Perinatal Mortality Rate 1962-1967*

	Breech Presentation	Cephalic Presentation
Total number of deliveries weighing over 2,500 g	124	5,928
Perinatal mortality (per cent)	4.91 (6/124)	1.13 (67.5/928)
Corrected mortality (per cent)	4.04 (1/24)	1.05 (62.5/928)

Table IV. *Attempts at Version*

	Number of patients	Per Cent	Number of Attempts at Version
Successful	240	81.1	262
Unsuccessful	56	18.9	8
Total	296	100.0	340

During the period 1962-1967 the incidence of breech delivery was 2.05 per cent (Table II)

The difference between the incidence of breech delivery when version was not performed and that when version was performed is 1.10 per cent. The decrease is statistically significant ( $p < 0.001$ ). The perinatal mortality rate for cephalic presentations over 2,500 g in the last 5-year period was 1.13 per cent. The perinatal mortality rate for the 124 deliveries of breech presentations was 4.91 per cent (Table III)

One of the six foetal deaths was due to a lethal malformation (anencephaly). The causes of the other foetal deaths were prolapse of the cord (2), intracranial haemorrhage (1) and unknown (2). The corrected mortality rate is 4.04 per cent (Table III). For cephalic presentations the corrected mortality rate is 1.05 per cent.

340 attempts at external version were performed in 296 patients. Two, three or four attempts were made if version was not successful or if the presentation reverted after a successful version. The results are illustrated in Table IV.

In the remaining 68 cases of breech deliveries attempts at version were not performed. 31 patients were admitted to the Department in labour without any antenatal care at the Department. 32 patients had a cephalic presentation at the antenatal clinic six to three weeks before term, but there may be a possibility of incorrect diagnosis since no radiographs were taken. Spontaneous version to a breech presentation is also a possibility. In 5 cases external version was contraindicated. 2 patients had antepartum haemorrhage and in 3 patients elective Caesarean section was planned because of the breech presentation in association with previous classical Caesarean sections (2) and pelvic contraction (1). Previous lower segment Caesarean section was not considered as a contraindication to the performance of external version.

In 3 cases external version was followed by complications and one of these resulted in foetal death.

IV provides, 28 years old, 4 weeks before the expected date of delivery the patient was found to have breech presentation. External version was performed 3 days after the version foetal movements disappeared and labour

began the next day. At this time foetal heart sounds could not be heard. Amniocentesis was performed and the liquor amnii was heavily blood-stained. Because of continuous bleeding a lower segment Caesarean section was performed. The uterus was very hard with subserous haemorrhages (Couvelair-uterus). The infant (weight 4 100 g) was macerated and there was a total abruption of the placenta.

In two cases external version was followed by rupture of the membranes and onset of labour. In both cases the infants were delivered as a vertex presentation. The birthweights were 3 200 g and 3,300 g and both had a high Apgar Score.

The foetal mortality rate due to version is in this report 0.38 per cent (1 out of 262 versions).

### *Conclusion*

The present series of single births weighing more than 2,500 g shows that the perinatal mortality rate for delivery of breech presentations is about four times as great as for delivery of cephalic presentations.

By performing prophylactic external cephalic version the incidence of breech delivery among infants weighing more than 2 500 g is lowered from 3.15 per cent to 2.05 per cent although, for different reasons, version was not attempted in a great many of the breech presentations.

The foetal loss due to external version was 0.38 per cent.

The corrected mortality rate for delivery of a cephalic presentation is 1.05 per cent.

The corrected mortality rate for delivery of a cephalic presentation after external version is 1.43 per cent. The mortality rate for delivery of a breech presentation is about 3 times as great.

The conclusion is that there is a need for prophylactic external cephalic version in order to reduce the foetal loss which would otherwise result from breech delivery.

### **SUMMARY**

In a retrospective study infants weighing more than 2,500 g the influence of prophylactic external cephalic version is investi-



gated. It is shown that by performing external version the incidence of breech delivery decreases by 11 per cent, which is statistically significant.

The corrected perinatal mortality rate for delivery of breech presentations is about 3 times as great as the mortality rate for external version followed by delivery of a cephalic presentation.

#### REFERENCES

- Brasher N A and Townsend, L J *Obst. & Gynec Brit Emp.* 67 668 1960  
Bishop E H Israel S L and Brice C C *Obst. & Gynec* 26 628 1965  
Brashear A. *Acta Obst et Gynec. Scandinav* 35 355 1956  
Cassart D E and Dodge S M. *Am. J Obst. & Gynec.* 27 517 1934  
Duffy G J *Obst. & Gynec Brit. Emp.* 59 841 1952  
Fell M R *The Lancet* 2 364 1953  
Fischer-Rasmussen W and Trolle D *Acta Obst. et Gynec. Scandinav* 46 Suppl 9 1957  
Friedlander D *Am J Obst. & Gynec.* 95 906 1956  
von Frunze B *Nordisk Medicin* 58 1830 1957  
Greenhill J P *Obstetrics* W B Saunders Philadelphia & London 1,th Ed 1963  
Johels P *Acta. Chir. et Gynec. Fenniae* 3 138 1949  
Kilhel P *Acta Obst et Gynec. Scandinav* 33 360 1954  
Morgan H S *Clin Obst. & Gynec.* 5 1009 1952  
Olav J and Pesse N *Acta Obst et Gynec. Scandinav* 30 Suppl 7 1950  
Vortex C K *J Obst. & Gynec Brit Emp* 52 417 1945

Received on April 3 1968

## OSTEOGENESIS IMPERFECTA

A Report of a Case of the Congenital form

BY

JOHANNES E. BOCK

Osteogenesis imperfecta is a rare disorder characterized by abnormal fragility of the bones resulting in fractures after minimal trauma, blue sclerotics because of thinness of the fibrous tissue, deafness because of otosclerosis, hyperlaxity of the ligaments, and deficient formation of dental enamel.

In 1835 Lobstein described the disease and called it osteoparathyrosis idiopathica (Lobstein's disease). In 1849 Vrolik described the congenital form and called it osteogenesis imperfecta. Looser (1906) concluded on the basis of histological similarities between Lobstein's and Vrolik's disease that they were two forms of the same disturbance although of different severity. This is still accepted to-day with only a few modifications (Schedorff 1949 Smuts 1961). The congenital form, osteogenesis imperfecta congenita, is usually the more severe with multiple fractures in the newborn or even in utero. The late form, osteogenesis imperfecta tarda, is found in infants, adolescents, or adults. This report concerns the congenital form of the disease.

Little is known about the aetiology. Until lately the basic disorder was thought to be a generalized inability of the osteoblasts to make enough lamellar bone (Rubin 1964). Recent investigations (Jett 1966 Lee 1964) employing a quantitative histological measuring technique with tetracycline as a marker seem to show that the only bone disorder is an inability to lay down periosteal bone. The result of this disturbance in bone formation is an irregular bone tissue with the Haversian systems more or less missing. The lamellae of the compact bone and trabeculae of the spongy bone are also poorly developed. The

bones are, therefore brittle and have a very thin cortex. The diaphysis of the long bones is deficient and slender while growth and differentiation of the epiphysis remains normal. The disorder involves bones of cartilaginous as well as membranous origin. The calvarium is very thin and in severe cases it consists of a membranous bag with isolated islands of bone tissue.

The essential radiological findings in children with osteogenesis imperfecta congenita are a thin bony cortex and a relatively large medullary cavity (Sarma 1960). The long bones have a slender shaft widening abruptly towards the epiphysis. Fractures can be seen in any part of the skeleton. They tend to heal with normal speed and often plenty of callus is found at the fracture site. The skull shows a mosaic appearance due to the presence of numerous islands of bone tissue. Skull fractures are common too. The vertebral column is often deformed (kyphosis or scoliosis) because of compression fractures and protrusion of the discs into the vertebral bodies. The disease is well advanced before birth and may be identified on prenatal X-ray films (Posner Goldman, 1957).

Genetic studies of the disease (Seedorff 1949 Smits 1961 Sarma 1960) show that the tarda variety is hereditary with dominant autosomal transmission. However there are different points of view about the congenital variety. Of Seedorff's (1949) 180 cases there are only 7 cases of the congenital type. In 6 of these cases no signs of the disease were found in the parents. In the seventh case the mother suffered from osteogenesis imperfecta tarda. Seedorff (1949) found 6 cases in literature in which a mother with osteogenesis imperfecta tarda gave birth to an infant affected by osteogenesis imperfecta congenita and he used these 6 cases and his own case to show that the congenital type is hereditary too. He believes that osteogenesis imperfecta congenita arising from normal parents is a product of a dominant mutation of certain genes. Others believe that osteogenesis imperfecta congenita is carried by a recessive gene (Gates 1946) or is not inherited (Funk 1940).

Biochemical investigations reveal normal serum calcium and phosphorus levels. The alkaline phosphatase is raised in some cases, but not in all (Sharma 1965).

## OSTEOGENESIS IMPERFECTA

A Report of a Case of the Congenital form

BY

JOHANNES E. BOCK

Osteogenesis imperfecta is a rare disorder characterized by abnormal fragility of the bones resulting in fractures after minimal trauma blue sclerotics because of thinness of the fibrous tissue deafness because of otosclerosis hyperlaxity of the ligaments, and deficient formation of dental enamel.

In 1835 *Lobstein* described the disease and called it osteoparathyrosis idiopathica (*Lobstein's disease*). In 1849 *Vrolik* described the congenital form and called it osteogenesis imperfecta. *Looser* (1906) concluded on the basis of histological similarities between *Lobstein's* and *Vrolik's* disease that they were two forms of the same disturbance although of different severity. This is still accepted to-day with only a few modifications (*Schedorff* 1949 *Smids* 1961). The congenital form, osteogenesis imperfecta congenita is usually the more severe with multiple fractures in the newborn or even in utero. The late form, osteogenesis imperfecta tarda, is found in infants adolescents or adults. This report concerns the congenital form of the disease.

Little is known about the aetiology. Until lately the basic disorder was thought to be a generalized inability of the osteoblasts to make enough lamellar bone (*Rubin* 1964). Recent investigations (*Jett* 1966 *Lee* 1964) employing a quantitative histological measuring technique with tetracycline as a marker seem to show that the only bone disorder is an inability to lay down periosteal bone. The result of this disturbance in bone-formation is an irregular bone tissue with the Haversian systems more or less missing. The lamellae of the compact bone and trabeculae of the spongy bone are also poorly developed. The

Fig. 2.



Fig. 3





Fig. 1

The incidence of the congenital form is not known. *Posner and Goldinan* (1957) state that osteogenesis imperfecta congenita is seen only once in 40 000 deliveries. *Freda et al* (1961) collected 90 cases from the literature. They say 1 in 21 000. Out of *Seedorff's* (1949) 180 cases only 7 were of the congenital type. *Campbell* (1966) has seen only one case in 15 000 deliveries.

### Case Report

AKK (Journ. 1063/67-8 Obst.) was a 34-year old woman gravida I para I with a 13 year history of infertility. In 1966 she had an operation for a toxic thyroid adenoma. The family history showed no evidence of bone diseases. Her last menstrual period was at the beginning of February 1967.

Fig. 2



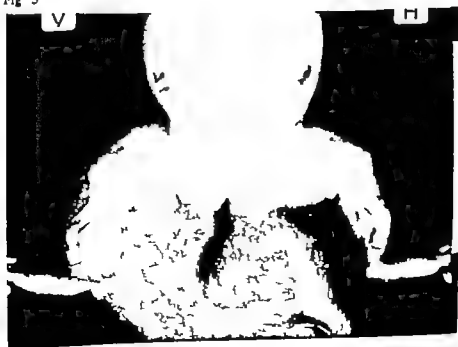
Fig. 3





Fig 4

Fig 5





Four weeks prior to the expected date of confinement abdominal and vaginal examination revealed breech presentation. External version without anaesthesia was attempted and two days later a cephalic presentation was found. On October 28, 1967 the patient was admitted in early labour. Examination again revealed a breech presentation. Because of the age and infertility of the patient and the breech presentation Caesarian section was performed. The puerperium was normal except for an umbilical rash.

#### *Examination of the baby showed.*

Weight 2500 g, length 42 cm. Respiration began within half a minute and there was no cyanosis. The infant developed an increasing respiratory rate (80) with paradoxical respiratory movements of the left part of the chest. The head appeared to be slightly enlarged. The calvarium was very soft and on palpation multiple bone islands could be felt. The eyes were slightly prominent with blue discolouration of both sclerotics. Both upper and lower limbs were deformed with crepitation in many places. The limbs were rather short (Fig 1)

#### *The radiological findings were*

Fig 2 A slightly hydrocephalic skull with mosaic appearance but no signs of fracture

Fig 3 X-ray films of the left arm showed a recent fracture of the shaft of the humerus and fracture of the radius and ulna with abundant callus.

Fig 4 An X-ray film of the right arm showed fractures at the upper and lower ends of the shaft of the humerus and fractures of both radius and ulna at the middle of the shaft.

Fig 5 An X-ray film of the legs showed fractures of both the femur and the tibia on both sides. Diffuse halisteresis was present too.

The baby died when 8 days old

A macroscopic examination showed very brittle bones with multiple fractures recent and old. Both sclerotics were thin and blue. No other malformations were present. A macroscopic examination showed extremely defective ossification both in cartilage and membranous bones. Only a few Haversian systems were present in the compact layer and the spongy bone appeared cystic in character. The calvarium was thin membrane of connective tissue with irregular osseous plates. The sternum was found consisting only of hyaline cartilage without ossification (T Lund, Aarhus)

#### *Comment*

This is a classical case of the congenital form of osteogenesis imperfecta. The parents were normal and the disease may be the result of a mutation. Four weeks before term external version of a breech presentation was performed. This version may per



Fig 4

Fig 5



## GLUCOSE-6-PHOSPHATASE IN HUMAN ENDOMETRIUM

BY

P. A. ÖCKERMAN

Glucose-6-phosphatase is an enzyme tightly bound to the endoplasmatic reticulum (microsomes after homogenization and centrifugation) (Hers and De Dupe 1950)

In man it is active in liver kidneys (Cori and Cori 1952) and jejunal mucosa (Öckerman 1964 and 1965). Although the existence of glucose-6-phosphatase has been suggested in several other tissues there is no convincing evidence of the presence of a specific microsomal enzyme in tissues other than liver kidneys and jejunal mucosa. Among the tissues studied is endometrium. Hughes et al. (1963) speculated on the function of glucose-6-phosphatase in human endometrium, and found a cyclic variation of the activity with the functional state of the tissue. However these authors only measured total glucose-6-phosphate splitting activity without characterizing the activity. Such an assay is totally unsatisfactory as a specific measure of glucose-6-phosphatase activity because other phosphatases, e.g. acid and alkaline phosphatases may have a considerable action on glucose-6-phosphate at pH 6.0-6.5 even in the absence of specific glucose-6-phosphatase (De Dupe et al. 1949 Öckerman 1965).

A highly sensitive method for the assay of glucose-6-phosphatase was recently presented (Öckerman 1967). This method greatly facilitated the study of phosphatase activities in human endometrium. The results of such a study are now presented.

haps have been responsible for some of the fractures. The clinical radiological and histological findings are all characteristic.

### SUMMARY

A case of osteogenesis imperfecta congenita is presented.

### REFERENCES

- Campbell J M. *The Medical Journal of Australia* 1 584 1966  
Freda V J Vosburgh G J Libert C D. *Obstetrics and Gynecology* 18 525 1961  
Funk P. *Schweitz Med. Wochr* 70 473 1940  
Gates R. R. *Human Genetics*. Macmillan, New York 2 765 1946  
Jett S Rarnser J R. Frost H M Vallenueva A. R. *Archives of Pathology* 81 112, 1956  
Lee W R. *Calcified Tissues* University of Liège 451 1954  
Lobstein J F. *Lehrbuch der Pathologischen Anatom.* Stuttgart 2 1 9 1835  
Looser E. *Mitt Grenzgeb Med. Chr* 15 161 1906  
Posner A. C Goldman J A. *Am J Obst. & Gynec.* 73 1143 1957  
Rubin P. *Dynamic Classification of Bone Dysplasias* Year Book Publications Inc. Chicago 314 1954  
Sarma Vishnu. *British Medical Journal* 2 1855 1960  
Seedorff K. S. *Osteogenesis Imperfecta* Munksgaard Copenhagen 1949  
Sharma N L. Singh R. N Anand J S. *Indian Pediatrics* 2 281 1955  
Smdrs G. *Osteogenesis Imperfecta in Sweden*. Norstedts. Stockholm 1951  
Vrolik W. *Tabulae ad Illustrandum Embryogenesin Hominis et Mamaliaum, tam Naturalem quam Abnormem*. Leipzig 1849

Received on April 3 1968

After 0 20 40 and 60 min. of incubation 0.1 ml of 10% trichloroacetic acid and 0.2 ml of water were added. The tubes were centrifuged and analysis was performed on 0.2 ml of the supernatant to which 0.3 ml of water 2.5 ml of malachite green and 0.1 ml of Tween 20 had been added.

#### *Fractionation of subcellular particles*

Fresh, unfrozen tissues were weighed, homogenized as described above and centrifuged in a Spinco Model L ultracentrifuge (Beckman-Spinco, Fullerton) in a swing-out rotor SW 39 L. The following fractions were collected: fraction N (625 g min unbroken cells and nuclei) M (30,000 g min mitochondrial fraction), L (250,000 g min lysosomal fraction) Mc (2,160,000 g min microsomal fraction) and S (Supernatant after 2,160,000 g min). The g minutes during acceleration and deceleration are included in the figures given. The system was devised (P. A. Öckerman, unpublished studies) for liver tissue and gave a distinct separation of acid phosphatase (as a marker for lysosomes) from specific glucose-6-phosphatase (as a marker for microsomes). The calculation of the results was as follows. The total activity of each enzyme was calculated for each fraction and expressed as a percentage of the activity of the enzyme in question in the whole homogenate. The same calculation was made for the protein content in each fraction and the value for the percentage enzyme activity was divided by the value for the percentage protein content. The figure thus obtained was called relative specific activity.

#### *Aggregation at pH 5*

To aggregate and concentrate microsomes the technique described by De Duve *et al.* (1949) was used. This technique involved incubation at pH 5.0 and 0°C for 15 min, centrifugation and suspension of the sediment in 0.25 M sucrose in 0.001 M EDTA (pH 7.0).

#### *pH-dependency*

The buffers used to study the enzyme activities as a function of pH for each experiment are described in the figures. The pH of

## Material and Methods

### Chemicals

These were as described earlier (Öckerman 1967)

### Patients

Endometrium was obtained from women aged 22–45 subjected to curettage usually because of menstrual irregularities. No patient with cancer of the endometrium was included. The day of the cycle was variable

### Preparation of the tissue

The curettings were immediately blotted on gauze to remove blood and then frozen on solid CO<sub>2</sub>. Tissues to be used for centrifugal separation of subcellular particles were chilled in ice and centrifugation was started within one hour. The tissue for chemical analysis was rapidly weighed and homogenized in 9 to 99 volumes of 0.25 M sucrose in 0.001 M EDTA (pH 7.0) in an all glass Potter Elvehjem homogenizer. The tissue was chilled in an ice bath throughout the procedure.

### Enzyme assays

Glucose-6-phosphatase was measured as described earlier (Öckerman 1967) using both glucose-6-phosphate (G6P) and  $\beta$ -glycerophosphate ( $\beta$ GLP) as substrates. Incubation times were 0, 60, 90 and 120 min. The final volume read in the photometer was 3.1 ml. The difference between the liberation of phosphate from G6P and that from  $\beta$ GLP was used to calculate the activity of glucose-6-phosphatase. This must not be confused with total G6P splitting activity.

Acid phosphatase was measured in the following way using the same assay principle as for glucose-6-phosphatase. 0.02 ml samples of homogenate usually prepared in 0.1% Triton X 100, 0.01 ml of 0.4 M acetate buffer (pH 5.0) and 0.02 ml of 0.5 M  $\beta$ GLP were incubated together at 37° in 0.4 ml stoppered plastic tubes (Beckman Spinco, Fullerton) within larger centrifuge tubes as earlier described for glucose-6-phosphatase (Öckerman 1967).

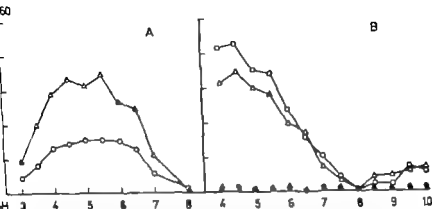


Fig. 1 Effect of pH on phosphatase activities in whole tissue homogenates. A Case 3 (Tab. I) Buffers: acetate (pH 3.0-5.5) imidazole (pH 6.0-8.0) Substrates, O—O G6P  $\Delta$ — $\Delta$   $\beta$ GLP Tissue homogenized 1/100 in 0.25 M sucrose 0.001 M EDTA (pH 7.0) Incubation time 2 hours. Zero minute values deducted. B. Case 8 (Tab. I) Buffers: acetate-borate-cacodylate according to de Duze *et al.* (1949). Substrates as in A.  $\bullet$ — $\bullet$  Tissue boiled for three minutes before assay G6P as substrate.  $\Delta$ — $\Delta$  Boiled tissue,  $\beta$ GLP as substrate Tissue homogenized 1/25 in 0.25 M sucrose 0.001 M EDTA (pH 7.0) with 0.1% Triton X 100 Incubation time 1 hour Zero minute values deducted.

case with higher activity on G6P (Fig. 1). No difference between the pH-curves for the two substrates was apparent in either case. The maximum activity on both substrates was noted at pH 4.5-5.0. The non-enzymatic liberation of phosphorus was negligible at all pH levels as evidenced from the experiment with boiled tissue homogenate.

#### Effect of pH on aggregated tissue fractions

Tissue homogenates were aggregated at pH 5.0 and 0°C and the sediment was suspended in sucrose-EDTA. This treatment diminished the phosphatase activity on  $\beta$ GLP more than that on G6P. The resulting pH-curves (Fig. 2) indicated the existence of glucose-6-phosphatase activity at optimum pH 5.5-6.5. This was

Table I Phosphate-Liberating Activity in Human Endometrium on Glucose-6-Phosphate (G6P) and  $\beta$ -Glycerophosphate ( $\beta$ GLP) All Values in  $\mu$ mole P/g Wet Weight/Min

	Substrate Used		Difference
	G6P	$\beta$ GLP	
1	1.03	1.53	-0.50
2	1.97	4.67	-2.70
3	0.83	1.48	-0.65
4	0.73	2.85	-2.12
5	1.51	0.53	+0.98
6	0.97	0.48	+0.49
7	0.86	0.52	+0.34
8	0.83	0.75	+0.08
9	0.75	0.46	+0.29
10	0.60	0.50	+0.10
11	0.68	0.25	+0.43

the incubation mixture was measured to ascertain that no deviations from the expected pH occurred.

#### Protein assay

This was performed according to Lowry *et al* (1951)

### Results

#### Normal range of phosphatase activities

The phosphate-liberating activity at pH 6.5 on G6P and  $\beta$ GLP of human endometrium is shown in Table I. In some cases  $\beta$ GLP was split at a higher rate than G6P. In these cases there existed no glucose-6-phosphatase activity when calculated as described previously and a negative value was obtained for the difference G6P splitting minus  $\beta$ GLP splitting activity. In other cases a positive value was obtained and thus the existence of a glucose-6-phosphatase as defined in this communication was indicated.

#### Effect of pH on whole tissue phosphatases

The effect of pH on the liberation of phosphate was studied in one case with higher activity on  $\beta$ GLP than on G6P and in one



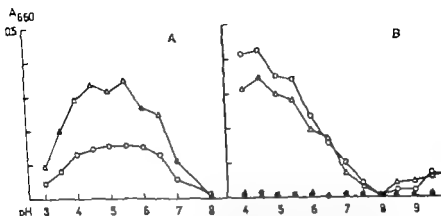


Fig. 1 Effect of pH phosphatase activities in whole tissue homogenates. A Case 3 (Table I). Buffers: acetate (pH 3.0-5.5) imidazol (pH 6.0-8.0). Substrates:  $\bigcirc$ - $\bigcirc$  G6P  $\triangle$ - $\triangle$   $\beta$ GLP. Tissue homogenized 1/100 in 0.25 M sucrose 0.001 M EDTA (pH 7.0). Incubation time 2 hours. Zero minute values deducted. B Case 8 (Tab. I). Buffers: acetate-borate-cacodylate according to de Druze et al (1949). Substrates as in A.  $\bullet$ - $\bullet$  Tissue boiled for three minutes before assay. G6P as substrate  $\triangle$ - $\triangle$  Boiled tissue,  $\beta$ GLP as substrate. Tissue homogenized 1/25 in 0.25 M sucrose 0.001 M EDTA (pH 7.0) with 0.1% Triton X 100. Incubation time 1 hour. Zero minute values deducted.

case with higher activity on G6P (Fig. 1). No difference between the pH-curves for the two substrates was apparent in either case. The maximum activity on both substrates was noted at pH 4.5-5.0. The non-enzymatic liberation of phosphorus was negligible at all pH levels as evidenced from the experiment with boiled tissue homogenate.

#### Effect of pH on aggregated tissue fractions

Tissue homogenates were aggregated at pH 5.0 and  $\square$  C and the sediment was suspended in sucrose-EDTA. This treatment diminished the phosphatase activity on  $\beta$ GLP more than that on G6P. The resulting pH-curves (Fig. 2) indicated the existence of glucose-6-phosphatase activity at optimum pH 5.5-6.5. This was

Table I. Phosphate-Liberating Activity in Human Endometrium on Glucose-6-Phosphate (G6P) and *p*-Glycerophosphate (*p*GLP) All Values in mole P/g Wet Weight/Min

	Substrate Used		Difference
	G6P	<i>p</i> GLP	G6P <i>p</i> GLP
1	1.03	1.53	-0.50
2	1.97	4.67	-2.70
3	0.83	1.48	-0.65
4	0.73	2.85	-2.12
5	1.51	0.53	+0.98
6	0.97	0.48	+0.49
7	0.85	0.52	+0.34
8	0.83	0.75	+0.08
9	0.75	0.46	+0.29
10	0.80	0.50	+0.30
11	0.68	0.25	+0.43

the incubation mixture was measured to ascertain that no deviations from the expected pH occurred.

#### Protein assay

This was performed according to *Loury et al* (1951)

### Results

#### Normal range of phosphatase activities

The phosphate-liberating activity at pH 6.5 on G6P and *p*GLP of human endometrium is shown in Table I. In some cases *p*GLP was split at a higher rate than G6P. In these cases there existed no glucose-6-phosphatase activity when calculated as described previously and a negative value was obtained for the difference G6P splitting minus *p*GLP splitting activity. In other cases a positive value was obtained and thus the existence of a glucose-6-phosphatase as defined in this communication, was indicated.

#### Effect of pH on whole tissue phosphatases

The effect of pH on the liberation of phosphate was studied in one case with higher activity on *p*GLP than on G6P and in one

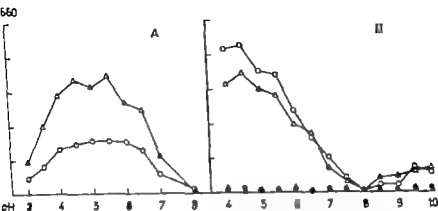


Fig. 1 Effect of pH on phosphatase activities in whole tissue homogenates. A Case 3 (Table I) Buffers: acetat (pH 3.0-5.5) imidazol (pH 6.0-8.0) Substrates,  $\bigcirc$ - $\bigcirc$  G6P  $\triangle$ - $\triangle$   $\beta$ GLP Tissue homogenized 1/100 in 0.25 M sucrose 0.001 M EDTA (pH 7.0). Incubation time 2 hours. Zero substrate values deducted B: Case 8 (Tab. I) Buffers: acetate-borate-cacodylate according to de Duve et al (1949) Substrates as in A.  $\bullet$ - $\bullet$  Tissue boiled for three minutes before assay G6P as substrate.  $\blacktriangle$ - $\blacktriangle$  Boiled tissue,  $\beta$ GLP as substrate Tissue homogenized 1/25 in 0.25 M sucrose 0.001 M EDTA (pH 7.0) with 0.1% Triton X 100. Incubation time 1 hour Zero substrate values deducted

case with higher activity on G6P (Fig. 1). No difference between the pH-curves for the two substrates was apparent in either case. The maximum activity on both substrates was noted at pH 4.5-5.0. The non-enzymatic liberation of phosphorus was negligible at all pH levels as evidenced from the experiment with boiled tissue homogenate.

#### Effect of pH on aggregated tissue fractions

Tissue homogenates were aggregated at pH 5.0 and B C and the sediment was suspended in sucrose-EDTA. This treatment diminished the phosphatase activity on  $\beta$ GLP more than that on G6P. The resulting pH-curves (Fig. 2) indicated the existence of glucose-6-phosphatase activity at optimum pH 5.5-6.5. This was

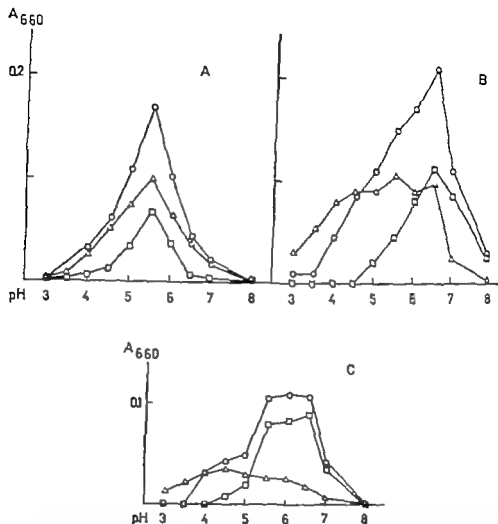


Fig. 2 Effect of pH on phosphatase activities in aggregated fraction of homogenate (see METHODS) A. Case 4 Substrates: O—O G6P  $\Delta$ — $\Delta$  pGLP  $\square$ — $\square$  phosphate liberation from G6P minus that from pGLP Homogenization buffers and incubation time as in Fig 1A. Zero minute values deducted. B. Case 9 Legend as in A C. Case 6 Legend as in A.

true both for Case 4 in whom direct analysis on whole tissue homogenate showed no glucose-6-phosphatase (G6P pGLP) activity (Table I) and also for Cases 6 and 9 in whom such activity was noted in whole tissue homogenates.

% OF INITIAL  
ACTIVITY

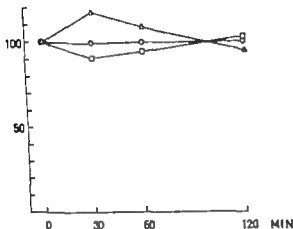


Fig 3 Stability of phosphatase activities at 37° C in an aqueous homogenate. Tissue homogenate from Case 6 1/50 in water was incubated at 37° for various times up to 2 hours. Phosphate assays were subsequently performed as described in METHODS after addition of sucrose-EDTA.

- phosphate liberation from G6P  
 △—△ phosphate liberation from  $\beta$ GLP  
 □—□ glucose-6-phosphatase (G6P  $\beta$ GLP)

#### *Stability of phosphatase activities at 37° C*

When a tissue homogenate in water was treated at pH 5 and 37° C for 10 min the phosphatase activity on both G6P and  $\beta$ GLP was stable (Table II). Stability was also noted for the phosphatase activities in a homogenate in water incubated at 37° C for various times up to 2 hours (Fig. 3).

#### *Localization of phosphatases in subcellular particles*

The fractionation of subcellular particles in the ultracentrifuge appeared to be a further means of partly separating the phosphatase activities on G6P and  $\beta$ GLP. In the experimental results recorded in Fig. 4 A it is seen that the relative specific activity

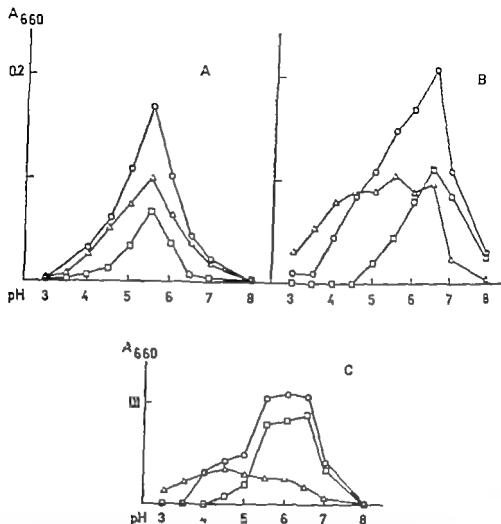


Fig. 7 Effect of pH on phosphatase activities in aggregated fraction of homogenate (see METHODS) A. Case 4 Substrates: O—O G6P  $\Delta$ — $\Delta$  pGLP  $\square$ — $\square$  phosphate liberation from G6P minus that from pGLP Homogenization, buffers and incubation time as in Fig. 1A. Zero minute values deducted. B. Case 9 Legend as in A. C. Case 6 Legend as in A.

true both for Case 4 in whom direct analysis on whole tissue homogenate showed no glucose-6-phosphatase (G6P, pGLP) activity (Table I) and also for Cases 5 and 9 in whom such activity was noted in whole tissue homogenates.

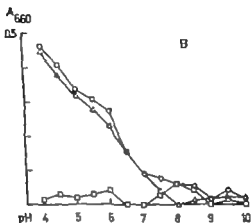


Fig. 4 B: Effect of pH on phosphatase activities in the lysosomal fraction. Buffers as in Fig. 1 B.

- phosphate liberation from G6P  
 △—△ phosphate liberation from βGLP  
 □—□ glucose-6-phosphatase (G6P/βGLP)

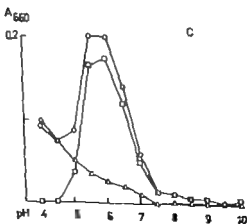


Fig. 4 C. The same experiment on the microsomal fraction.

Table II Stability of Phosphatase Activities at pH 5 and 37°C

Tissue homogenate from Case 9 1/10 in water was titrated to pH 5 with HCl and incubated for 10 min at 37°C. Phosphatase activities were measured at pH 6.5 Before and After this Incubation. Incubation Times 0 10 20 30 min. All values in  $\mu\text{mole P/g wet weight/min.}$

Substrate	Direct Assay	Assay after Treatment at pH 5 and 37°C
G6P	0.75	0.78
$\beta$ GLP	0.46	0.48

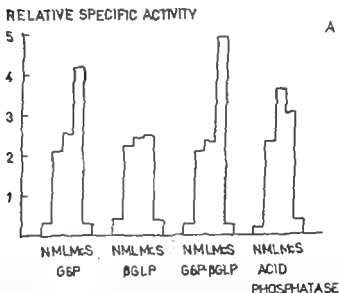


Fig. 4 Subcellular localization of phosphatase activities. Unfrozen tissue homogenate from Case 11 1/25 in 0.25 M sucrose in 0.001 M EDTA (pH 7.0) was fractionated in the ultracentrifuge as described in METHODS. The sediments were suspended in sucrose-EDTA before the assays. In the assay of acid phosphatase Triton X 100 was added to a concentration of 0.1% (v/v). N=nuclear fraction, M=mitochondrial fraction, L=lysosomal fraction, Mc=microsomal fraction, S=supernatant.

Fig. 4 A. Relative specific activity in each fraction calculated as described in METHODS.



trium. Clearly no method of assay of this enzyme can be devised from these findings. Indeed, it is not clear whether any specific glucose-6-phosphatase activity exists even when the splitting at pH 6.5 is greater for G6P than for  $\beta$ GLP

#### *Effect of pH on aggregated tissue phosphatases*

The technique used to aggregate microsomes is not specific for these particles, but has been used earlier with success by the author (Ockerman 1965) to separate and purify glucose-6-phosphatase from alkaline phosphatase in intestinal mucosa. Although it seemed from the pH-curves from whole tissue homogenate that acid phosphatase was the principal phosphatase in endometrium, the aggregation technique was tried. The results clearly indicate that glucose-6-phosphatase is present in human endometrium provided this enzyme is defined as described previously in this paper. The effect of pH on the glucose-6-phosphatase (G6P  $\beta$ GLP) activity clearly differs from that on the  $\beta$ GLP splitting activity and also from that on the total G6P-splitting activity in whole tissue homogenates. These findings may be interpreted as indicating the existence of a specific glucose-6-phosphatase which may be similar to that in liver kidneys and jejunal mucosa. An alternate explanation might be a different pH-dependency of acid phosphatase according to whether G6P or  $\beta$ GLP is used as a substrate

#### *Stability of phosphatases activities at 37 C*

Glucose-6-phosphatase in liver kidneys and jejunal mucosa is very labile at 37 C when incubated at pH 5 or without substrate (De Duve et al 1949 Ockerman 1965). Therefore, the results found here for endometrial glucose-6-phosphatase, indicating a great stability of the total activity on G6P are unexpected, if any specific glucose-6-phosphatase exists in endometrium. At present no explanation for this apparent stability can be given.

#### *Localization of phosphatases in subcellular particles*

The results presented in this communication support the concept that glucose-6-phosphatase (G6P  $\beta$ GLP) activity has a different

of glucose-6-phosphatase (G6P  $\beta$ GLP) was maximal in the microsomal fraction. The  $\beta$ GLP splitting activity and acid phosphatase showed a different pattern with maximum activity in both the lysosomal and the microsomal fractions.

The effect of pH on phosphatase activities further illustrated this difference (Fig. 4 B and C) the glucose-6-phosphatase (G6P  $\beta$ GLP) activity being optimum at pH 6.0 in the microsomal fraction. The phosphatase activity on  $\beta$ GLP however was greater at more acid pH levels. In the lysosomal fraction much less glucose-6-phosphatase (G6P  $\beta$ GLP) activity was found with no measurable pH-optimum while the  $\beta$ -glycerophosphatase activity was most active at pH 4 or possibly even lower.

### Discussion

#### *Normal range of phosphatase activities*

No normal values for the activity of glucose-6-phosphatase in endometrium can be stated. This is because the activity on  $\beta$ GLP was often higher than that on G6P. If any specific glucose-6-phosphatase activity existed in these cases it would be concealed by the higher activity of other phosphatases on  $\beta$ GLP. Thus the method used for the assay and calculation of specific glucose-6-phosphatase may not give a true estimation of the enzyme activity if this is low compared with that of other phosphatases present. However no other method, except possibly the use of inhibitors (Di Bella *et al.* 1963) is at present available to measure specific glucose-6-phosphatase in the presence of other phosphatases. An attempt to use such inhibitors was unsuccessful, however (P. A. Öckerman, unpublished experiments).

The results very clearly demonstrate that it is inadequate to measure the total phosphate liberating activity on G6P and, as has been the practise call this glucose-6-phosphatase (Hughes *et al.* 1963). A large part of the activity thus measured comes from other phosphatases.

#### *Effect of pH on whole tissue phosphatases*

The pH curves on whole tissue homogenates further demonstrate the difficulty in measuring glucose-6-phosphatase in endome-

trium. Clearly no method of assay of this enzyme can be devised from these findings. Indeed, it is not clear whether any specific glucose-6-phosphatase activity exists, even when the splitting at pH 6.5 is greater for G6P than for  $\beta$ GLP

#### *Effect of pH on aggregated tissue phosphatases*

The technique used to aggregate microsomes is not specific for these particles but has been used earlier with success by the author (Ockerman 1965) to separate and purify glucose-6-phosphatase from alkaline phosphatase in intestinal mucosa. Although it seemed from the pH-curves from whole tissue homogenate that acid phosphatase was the principal phosphatase in endometrium, the aggregation technique was tried. The results clearly indicate that glucose-6-phosphatase is present in human endometrium provided this enzyme is defined as described previously in this paper. The effect of pH on the glucose-6-phosphatase (G6P  $\beta$ GLP) activity clearly differs from that on the  $\beta$ GLP splitting activity and also from that on the total G6P-splitting activity in whole tissue homogenates. These findings may be interpreted as indicating the existence of a specific glucose-6-phosphatase which may be similar to that in liver kidneys and jejunal mucosa. An alternate explanation might be a different pH-dependency of acid phosphatase according to whether G6P or  $\beta$ GLP is used as a substrate.

#### *Stability of phosphatases activities at 37 C*

Glucose-6-phosphatase in liver kidneys and jejunal mucosa is very labile at 37 C when incubated at pH 5 or without substrate (De Durs *et al.* 1949 Ockerman 1965). Therefore the results found here for endometrial glucose-6-phosphatase, indicating a great stability of the total activity on G6P are unexpected, if any specific glucose-6-phosphatase exists in endometrium. At present no explanation for this apparent stability can be given.

#### *Localization of phosphatases in subcellular particles*

The results presented in this communication support the concept that glucose-6-phosphatase (G6P  $\beta$ GLP) activity has a different

subcellular localization from that of acid phosphatase and the activity on  $\beta$ GLP at pH 6.5. The fact that the patterns of the latter two activities were very similar might indicate that the  $\beta$ GLP splitting activity at pH 6.5 reflects mainly acid phosphatase activity.

The pH-dependency of the activities measured in the lysosomal and microsomal fractions further support the idea that there exists in endometrium a glucose-6-phosphatase activity not identical with acid phosphatase. The fact that the relative specific activity of acid phosphatase was the same in both the lysosomal and the microsomal fractions indicates either contamination by lysosomes in the microsomal fraction or that the microsomes contain an enzyme active on  $\beta$ GLP at pH 4 as well as at pH 6.5. This enzyme may or may not be the glucose-6-phosphatase discussed here.

The results of the assays on subcellular particles give evidence against the alternative explanation put forward in the discussion of the aggregation experiments. Thus the different pH-curves found on aggregated material using G6P and  $\beta$ GLP respectively could not be caused by the same enzyme.

### *General Conclusions*

All the findings support the theory that there is in human endometrium a glucose-6-phosphatase which differs from acid phosphatase. This enzyme might have the same intracellular localization as the specific glucose-6-phosphatase in liver although there is no direct proof of this. The enzyme is more stable than the specific glucose-6-phosphatase in liver, kidneys and jejunal mucosa. It may be genetically the same enzyme as glucose-6-phosphatase in the other tissues but its properties are not identical. One means of elucidating the possible genetic identity of glucose-6-phosphatase in endometrium and liver, kidneys and jejunal mucosa would be by investigation of patients with glycogen storage disease type I who have a genetically determined deficiency of glucose-6-phosphatase in liver, kidney and jejunal mucosa (Cori and Cori 1952, Öckerman 1964). So far it has not been possible to obtain endometrium from such a patient.

## SUMMARY

Phosphatase activity in human endometrium was studied using glucose-6-phosphate (G6P) and  $\beta$ -glycerophosphate ( $\beta$ GLP) as substrates. When whole tissue homogenates were used the splitting of G6P was higher than that of  $\beta$ GLP in some patients. By studying the pH-dependency of the activities on G6P and  $\beta$ GLP no definite indication of the existence of a specific glucose-6-phosphatase was obtained.

By aggregating microsomes at pH 5.0 at 0°C and by ultra centrifugal fractionation of subcellular particles and assay of G6P and  $\beta$ GLP-splitting activities at various pH levels results indicated the existence, in the microsomal fraction, of a glucose-6-phosphatase not identical with acid phosphatase. This enzyme had its optimum activity at pH 5.5-6.5 and was stable at 37°C even at pH 5. It thus resembles in many but not all, respects the specific glucose-6-phosphatase known to exist in liver, kidney and jejunal mucosa.

*Acknowledgements*

The present investigation was supported by the Swedish Medical Research Council (grant No. K68-19X 2222). Skilful technical assistance was given by Mrs G. Lindfors and Miss M. Sjöland.

## REFERENCES

- Di Bella S, Richetto G and Pickleiri U. *Clin. Chim. Acta* 8: 788, 1953.  
Cori G T and Cori C F. *J Biol. Chem.* 199: 661, 1952.  
De Duve C, Berthet J, Hers H G and Dupret L. *Bull. Soc. Chim. Biol.* 31: 1242, 1949.  
Hers H G and De Duve C. *Bull. Soc. Chim. Biol.* 32: 20, 1950.  
Hughes H C, Jacobs R. D, Rubulis A and Huxley R. M. *Ann. J. Obst. Gynec.* 85: 594, 1963.  
Loury O H, Rosebrough N J, Farr A. L. and Randall R. J. *J Biol. Chem.* 193: 265, 1951.  
Ockerman P A. *Clin. Chim. Acta* 9: 151, 1964.  
Ockerman P A. *Biochim. Biophys. Acta* 11: 322, 1963.  
Ockerman P A. *Clin. Chim. Acta* 17: 201, 1957.

Received on March 21, 1968

## LIVER REACTION IN CONNECTION WITH ORAL CONTRACEPTIVE STEROIDS

BY

JOHAN BROHULT AND ANDERS WESTGREN

Many retrospective studies have shown that there is probably a causal connection between icterus and the use of peroral contraceptive steroids (Baines 1965 Larsson-Cohn 1965 Thulin and Nermark 1966). One of the purposes of this prospective study was to investigate how often the introduction of peroral contraceptives is followed by clinically important disturbances in the liver.

Rises in serum transaminases (Knutson *et al* 1965 Larsson-Cohn 1966 Orellana Alcalde and Dominguez 1966) and in serum ornithine carbamoyl transferase (S-OCT) (Brohult and Westgren 1965) have been observed after contraceptive steroids and an increase in bromsulphthalein retention has also been demonstrated after peroral contraceptives (Elsalo *et al* 1964 Larsson-Cohn 1967). OCT was considered to be a particularly suitable enzyme for this study since it occurs only in the liver and even a minor disturbance in this organ elicits a rise in S-OCT (Reichard 1962). Analyses were made of the S-OCT at specific intervals in order to elucidate how the liver reacts after the introduction of contraceptive steroids as well as to investigate whether any liver injury can be detected at an early stage.

### *Material*

The study was made on 127 women who applied to the gynecological clinic for peroral contraceptives. These women were selected as follows:

- 1 Only women with no liver diseases in the anamnesis were included.
- 2 Only clinically healthy women with a normal gynecological status were included.
- 3 All women with an initially pathologically elevated S-OCT ( $> 40$  nm) and/or S-GPT ( $> 40$  units) were excluded. This applied in 7 cases.
- 4 Women who missed one of the regular blood tests were excluded. This applied to 149 cases the test after one month being missed by 137 women and the test after three months by 12 women. Since so many women missed the test after one month unfortunately it became practically impossible to remind them all by letter or by telephone. Such reminders, were, however sent out each time someone missed one of the blood tests after 3 6 or 12 months.

The contraceptive tablets were prescribed in the ordinary manner the preparations being the four most commonly used in Sweden 1965 and 1966 ethinyloestradiol 0.05 mg, norethisterone 4 mg (Anovlar<sup>®</sup>) mestranol 0.1 mg, norethisterone 2 mg (Conhuten<sup>®</sup>) mestranol 0.075 mg, norethynodrel 5 mg (Enavid Mite<sup>®</sup>) and mestranol 0.075 mg, lynestrenol 2.5 mg (Lyndiol Mite<sup>®</sup>). Since no differences were found between these four groups they have been combined in the following account.

Having received their prescriptions, the women were sent to have a sample of blood taken at the laboratory the plan being to take further samples in 1 3 6 and 12 months time.

Of the 127 women included in the study 18 stopped using the preparation after about 3 months and 7 stopped after about 6 months. Of these 25 women who stopped using the preparations (cf Tables III and IV) 19 did so because of side effects (cf Fig. 4) and 6 for fear of side effects or because they wished to become pregnant.

Data concerning the 102 women who were followed throughout the study are given in Tables I II and Figs. 1-3. Their ages when treatment started varied between 18 and 47 years. These 102 women included seven who developed side effects towards the end of the year but who nevertheless agreed to continue with

the preparation for just under 12 months. Data on these seven women are given in Fig. 4 together with the 19 women who stopped because of side effects after 3-6 months.

### Methods

#### Sampling

Samples for the analyses of serum enzymes were taken on five occasions before the introduction of the contraceptive steroids, after 1 month of medication ( $\pm 1$  week) after 3 months ( $\pm 2$  weeks) after 6 months ( $\pm 3$  weeks) and after 12 months ( $\pm 6$  weeks).

#### Analyses

Recognized methods were used for the statistical calculations (Dixon and Massey 1957).

By reproducibility (coefficient of variation) is meant the error of the method calculated from duplicated determinations in per cent of the mean of the duplicate determinations

$$\frac{100 \sqrt{\frac{\sum d^2}{2n}}}{\bar{x}}$$

The OCT activity was determined by incubation of serum with citrulline carbamoyl  $^{14}\text{C}$  in arsenate buffer (Reichard 1964). The results are expressed in nanomoles (nm)  $^{14}\text{CO}_2$  liberated by 0.5 ml serum during two hours incubation under standard conditions. Normal value  $\leq 4$  nm (Beckman et al. 1966) i.e. 0.004 micromoles  $^{14}\text{CO}_2$ . Reproducibility 8 per cent.

GOT and GPT were determined by the NADH method (Karmen 1955) as modified by Ordell (1956). Normal value  $< 40$  Karmen units (Allgén 1962). Reproducibility 5 per cent.

Lactic dehydrogenase (LD) was determined by the method of Wróblewski and LaDue (1955). Normal value 100-300 Wróblewski units. One unit corresponds to a consumption of 0.53 micromoles per litre and minute. Reproducibility 4 per cent.



Bilirubin was determined by the method of Jendrassik and Gróf (1938) according to the modification of Nossli (1960) Normal value, <1.2 mg per 100 ml (Nossli 1960) Reproducibility 5 per cent.

The alkaline phosphatase activity in serum was determined by the method of Roos (1963) according to the modification of Kahl. Normal value 2-8 Buch & Buch units (Buch and Buch 1939) Reproducibility 6 per cent.

The thymol turbidity was determined according to MacLagan (1944) Normal value <4 MacLagan units. Reproducibility 4 per cent.

### Results

The 102 women who completed the study displayed a slight rise in the alkaline phosphatase activity in serum after one month ( $P < 0.05$ ). On no occasion were any significant changes detected in bilirubin, thymol turbidity or LD in serum (*cf* Table II). Two women who stopped taking the tablets because of icterus and itching (cases 93 and 108 *cf* Fig. 4) displayed moderately elevated serum bilirubin (3.2 and 4.7 mg per cent respectively).

In the 102 women who completed the study S-OCT (*cf* Table I A-C) S-GOT (*cf* Fig. 2) and S-GPT (*cf* Fig. 3) showed a significant rise after one month ( $P < 0.001$ ) and then successively dropped again (*cf* Fig. 1-3). The average levels of S-OCT after 1 month (4.8 nm), 3 months (3.7 nm), 6 months (3.3 nm) and 12 months (3.0 nm) are all significantly higher than the initial level (1.7 nm) with  $P < 0.001$ , 0.001, 0.01 and 0.05 respectively.

As will be seen from Fig. 1 the subjects were divided into three groups according to the course of S-OCT: group A (plain group) comprises those women whose serum-OCT did not exceed 4.0 nanomoles (nm) in any of the tests (Table I A); group B (hull group) comprises the women whose S-OCT exceeded 4.0 nm on one or several occasions but was found to have normalized (4.0 nm or below) after 12 months (Table I B); group C (rise group) finally comprises the women whose S-OCT exceeded 4.0 nm after 12 months (Table I C). The rises in S-OCT after 1 and 3 months are statistically significant in all

Table 1 A. *The 47 Women out of 102 with S-OCT Never Exceeding 40 Nanomoles*

Case	Initial 0	Months after Start			
		1	3	6	12
5	0.1	0.6	1.2	0.0	0.4
6	0.7	1.0	1.6	1.0	0.9
7	0.7	0.6	2.2	1.2	1.3
8	0.5	2.0	1.3	0.0	0.2
11	0.8	1.1	2.0	1.5	0.9
15	1.2	2.8	2.3	1.9	1.3
16	1.0	2.5	2.8	3.6	2.4
18	3.3	3.8	2.2	2.2	1.4
20	2.1	2.9	1.9	1.4	0.4
32	1.5	1.4	2.3	1.7	1.9
35	1.4	4.0	3.4	3.0	1.0
36	0.5	2.4	0.5	1.6	2.0
38	0.7	1.7	2.6	0.4	0.4
39	0.1	2.9	1.3	1.4	3.3
41	2.7	1.4	0.5	1.1	1.9
43	1.3	1.3	0.8	0.5	0.5
48	0.5	2.7	1.0	0.6	0.1
55	0.1	3.4	1.0	1.7	1.7
56	1.0	1.3	1.5	3.5	1.5
57	0.0	0.3	1.6	0.7	0.9
62	1.2	3.4	2.5	0.3	1.4
75	1.0	2.5	1.8	2.0	1.5
78	2.0	1.9	1.7	2.3	0.7
79	0.9	3.9	3.8	3.9	3.3
80	4.0	3.7	2.4	3.8	3.8
86	2.7	2.3	1.8	1.9	2.6
87	1.2	1.7	2.3	2.9	3.8
91	2.7	1.6	1.0	1.1	1.5
95	0.8	1.7	1.8	2.8	1.2
96	0.3	1.3	3.0	1.8	0.3
97	2.9	1.4	3.4	3.9	3.0
98	1.7	2.2	1.1	1.0	1.4
100	0.5	2.9	1.3	1.8	0.3
101	0.3	0.9	0.3	0.6	0.3
104	1.5	1.6	1.0	1.8	1.3
107	0.7	3.1	2.0	1.5	0.2
109	1.4	1.8	2.6	1.5	2.2
112	1.0	1.5	1.3	1.8	0.3
114	1.0	1.5	2.3	1.0	2.3
117	1.3	2.7	1.9	2.1	0.5
125	0.8	2.1	0.8	0.3	1.0
126	0.3	0.6	1.8	0.6	1.5
129	0.4	3.9	3.0	2.2	1.0
130	1.3	3.9	1.7	1.4	0.2
133	1.2	1.1	2.4	1.1	0.8
137	1.4	3.0	1.6	0.7	1.3
139	1.8	2.7	1.9	2.0	1.2
$\bar{x}$	1.2	2.1	1.8	1.6	1.3
SD	0.9	1.0	0.8	1.0	1.0
Range	0.0-4.0	0.3-4.0	0.3-3.8	0.0-3.9	0.1-3.8

Table 1 B The 37 Women out of 102 with S-OCT Exceeding 4.0 Nanomoles (nm) on One or Several Occasions but with S-OCT below 4.0 nm after One Year

Case	Initial 0	Months after Start			
		1	3	6	12
3	1.6	4.1	3.5	1.9	1.2
4	1.6	4.7	0.8	1.2	1.0
10	3.4	9.7	7.7	6.2	3.7
19	2.5	2.7	3.2	4.9	3.2
21	1.3	12.0	7.1	4.1	3.6
22	2.4	4.4	3.9	2.3	1.9
23	2.0	4.2	2.8	1.9	3.1
27	1.9	16.3	2.3	4.7	1.8
36	1.8	4.4	3.2	2.6	3.3
34	1.6	9.2	15.2	7.0	3.4
37	0.9	1.1	1.8	5.5	2.5
43	2.4	5.3	7.1	5.9	2.0
49	1.4	5.9	2.5	0.4	1.4
50	1.1	14.6	8.3	4.1	0.3
60	1.8	9.0	0.8	1.6	0.3
67	2.4	5.0	1.8	1.9	1.2
70	1.6	5.2	5.0	1.8	2.8
71	0.7	4.9	2.1	1.8	1.1
74	0.5	4.2	2.7	0.7	1.0
81	2.0	5.3	2.2	0.5	3.8
90	2.4	5.8	3.9	1.3	0.0
94	0.8	3.9	4.5	4.0	3.2
99	0.8	7.4	6.3	1.7	1.7
103	1.1	19.8	1.1	0.7	0.2
106	0.6	1.8	0.8	6.7	1.1
111	1.5	8.0	6.0	2.9	1.1
113	0.3	8.9	5.5	2.0	3.6
115	2.6	17.5	2.6	1.7	2.7
120	2.8	5.0	2.7	3.7	3.1
122	2.2	2.3	2.4	6.5	1.6
123	2.1	8.8	2.7	1.0	1.2
127	3.0	15.7	14.0	6.2	3.9
128	1.3	10.5	5.6	1.6	0.2
134	3.4	7.3	6.0	1.4	2.2
141	0.9	3.2	5.8	3.3	1.8
142	3.1	1.5	9.0	0.0	2.4
143	3.1	5.7	1.7	1.0	2.5
$\Sigma$	18	72	44	29	20
SD	0.9	4.6	3.3	2.1	1.2
Range	0.3-3.4	1.1-19.8	0.8-15.2	0.0-7.0	0.0-3.9

Table I C. *The 18 women out of 102 with S-OCT Exceeding 4.0 Nanomoles after One Year*

Case	Initial 0	Months after Start			
		1	3	6	12
12	0.9	5.8	6.2	4.5	7.2
17	2.8	7.4	9.5	6.6	9.8
23	2.5	2.3	2.7	38.9	74.5
25	1.3	5.6	8.8	10.2	13.6
33	3.8	7.5	5.5	5.1	7.1
44	3.5	9.1	8.0	8.8	10.7
46	4.0	10.3	7.3	3.0	6.2
52	0.8	3.8	3.2	2.1	4.3
58	3.8	9.4	4.6	6.7	11.3
63	2.6	8.9	12.1	9.6	16.3
69	2.5	10.1	18.0	14.3	16.5
73	3.7	5.4	14.6	1.9	4.7
83	2.8	3.6	4.0	7.6	6.1
84	1.0	12.4	3.2	5.4	5.2
105	3.9	7.7	9.8	18.0	8.5
116	4.0	1.1	1.9	4.3	4.2
132	1.2	5.9	7.8	4.5	6.8
135	2.0	4.2	4.1	2.6	5.3
$\bar{x}$	2.6	6.7	7.0	8.6	9.4
SD	1.2	3.0	4.4	8.7	5.4
Range	0.8-4.0	1.1-12.4	1.9-18.0	1.9-38.9	4.2-24.5

three groups ( $P < 0.001$ ) After 6 and 12 months however the rise is still significant only in group C ( $P < 0.01$ ) The initial level of S-OCT in group C is significantly higher than the initial levels in the other two groups ( $P < 0.01$ )

There were 5 women who stopped taking the tablets because of itching. They all displayed a pathological rise in S-OCT ( $> 4.0$  nm) when they stopped.

Side reactions that caused cessation of treatment with oral contraceptives after 3-12 months were observed in 26 of the 127 women (see Fig. 4)

An attempt was made to follow up the 149 women who missed one of the tests but it proved very difficult to obtain answers from them all. From the answers that were obtained however

Table II. Mean Serum Levels of OCT GOT GPT LD Bilirubin Alkaline Phosphatases and Thymol Turbidity in 102 Women Using Oral Contraceptive Steroids for One Year

Analysis	Initial	Months after Start			
		1	3	6	12
OCT (nanomoles)	1.7	4.8	3.7	3.3	3.0
GOT (Karmen units)	21	25	23	22	22
GPT (Karmen units)	20	32	27	24	23
LD (Wroblewski units)	208	213	205	208	206
Bilirubin (mg/100 ml)	0.47	0.53	0.49	0.52	0.50
Alkaline phosphatases (Bach and Bach units)	3.5	3.8	3.7	3.6	3.5
Thymol turbidity (MacLagan units)	1.0	1.1	1.0	1.1	1.1

It seems that roughly half did not turn up because they had stopped using the contraceptive in question, while the other half either had not wished or were unable to come to the hospital for a blood test. Of those who had stopped using oral contraceptives, roughly half had done so for fear of secondary effects about which they had read in the press while roughly half had done so because of secondary effects such as putting on weight, a feeling of tension in the breasts, breakthrough bleedings or mental complaints such as depression, anxiety and irritability. On the other hand, no serious secondary effects were found among these women. Some of them even had difficulty in remembering, or were unwilling to state why they had stopped using peroral contraceptives partly because the follow-up study was not conducted until one or two years after the contraceptive had been prescribed for the first time.

Table 1 C. *The 18 women out of 102 with S OCT Exceeding 4.0 Nanometers after One Year*

Case	Initial 0	Months after Start			
		1	3	6	12
12	0.9	5.8	6.2	4.5	7.2
17	2.8	7.4	9.5	6.6	9.8
23	2.5	2.3	2.7	38.9	74.5
25	1.3	5.6	8.8	10.2	13.6
33	3.8	7.5	5.5	5.1	7.1
44	3.5	9.1	8.0	8.8	10.7
46	4.0	10.3	7.3	3.0	6.2
52	0.8	3.8	3.2	2.1	4.3
58	3.8	9.4	4.6	6.7	11.3
63	2.6	8.9	12.1	9.6	16.3
69	2.5	10.1	18.0	14.3	16.5
73	3.7	5.4	14.6	1.9	4.7
83	2.8	3.6	4.0	7.6	6.1
84	1.0	12.4	3.2	5.4	5.2
105	3.9	7.7	9.8	18.0	8.5
116	4.0	1.1	1.9	4.3	4.2
132	1.2	5.9	7.8	4.5	6.8
135	2.0	4.2	4.1	2.6	5.3
$\bar{x}$	2.6	6.7	7.0	8.6	9.4
SD	1.2	3.0	4.4	8.7	5.4
Range	0.8-4.0	1.1-12.4	1.9-18.0	1.9-38.9	4.2-14.5

three groups ( $P < 0.001$ ) After 6 and 12 months however the rise is still significant only in group C ( $P < 0.01$ ) The initial level of S-OCT in group C is significantly higher than the initial levels in the other two groups ( $P < 0.01$ )

There were 5 women who stopped taking the tablets because of itching. They all displayed a pathological rise in S-OCT ( $> 4.0$  nm) when they stopped.

Side reactions that caused cessation of treatment with oral contraceptives after 3-12 months were observed in 26 of the 127 women (see Fig. 4)

An attempt was made to follow up the 149 women who missed one of the tests but it proved very difficult to obtain answers from them all. From the answers that were obtained, however

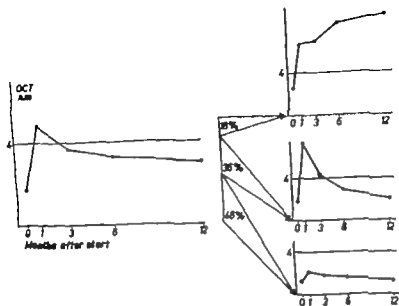


Fig 1 S-OCT in 102 women using oral contraceptive steroids for one year. The small figures represent the three groups based upon the course of S-OCT.

Table IV The 7 Women (out of 127) Who Stopped Using Oral Contraceptives after 6 Months

Case	Initial	Months after Start		
		1	3	6
9	3.2	5.2	8.6	7.7
42	2.0	3.3	1.8	2.1
54	1.2	8.0	3.0	0.8
68	0.6	1.5	2.9	0.1
92	2.1	3.2	4.8	3.4
111	1.3	6.0	5.7	5.4
118	2.3	4.5	2.7	3.2
Mean	1.8	4.5	4.2	3.2

Table III *The 18 Women (out of 127) Who Stopped Using Oral Contraceptives after 3 Months*

Case	Initial 0	Months after Start	
		1	3
13	1.2	0.8	1.1
24	1.1	0.1	1.4
29	0.5	11.0	.6
40	1.8	5.9	1.6
51	1.4	3.5	2.9
53	1.4	2.6	8.7
61	0.4	4.5	2.0
64	4.0	4.7	3.3
65	3.1	1.7	1.2
76	3.5	34.1	20.5
88	1.4	0.2	0.6
89	1.0	26.8	17.5
102	1.1	1.3	1.8
108	0.9	1.2	15.3
124	2.3	1.9	1.8
131	3.1	9.6	30.1
136	2.5	13.4	5.5
146	0.6	3.4	1.7
Mean	1.7	7.0	7.0

### Discussion

Treatment with contraceptive steroids places an additional load on the excretory function of the liver (Adlercreutz 1964 Eisalo *et al* 1965 Sherlock 1968) and induces changes in the metabolism of carbohydrates (Wynn and Doar 1966) lipids (Wynn *et al* 1966 Aurell and Cramér 1966) and proteins (Robertson 1967). Many cases of icterus after contraceptive steroids have been reported (*e.g.* Cullberg *et al* 1965 Orellana Alcalde and Domingue 1966) and it is considered that a relationship exists between peroral contraceptives and icterus (Baines 1965 Larsson-Colin 1965). One of the aims of the present study was to assess the incidence of clinically relevant effects on the liver. The 5 cases with itching and fatigue (2 of which also displayed icterus) all had high S OCT levels (*cf.* Fig. 4). The symptoms



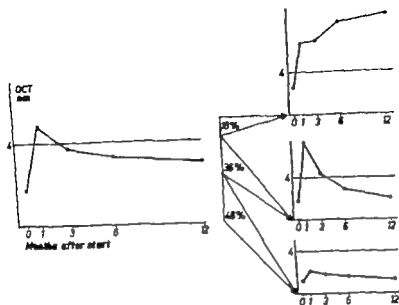


Fig 1 S-OCT in 102 women using oral contraceptive steroids for one year  
The small figures represent the three groups based upon the course of S-OCT

Table IV The 7 Women (out of 127) Who Stopped Using Oral Contraceptives after 6 Months

Case	Initial 0	Months after Start		
		1	3	6
9	3.2	5.2	8.6	7.7
42	2.0	3.3	1.8	2.1
54	1.2	8.0	3.0	0.8
68	0.6	1.5	2.9	0.1
92	2.1	3.2	4.8	3.4
93	1.3	6.0	5.7	5.4
118	2.3	4.5	2.7	3.2
Mean	1.8	4.5	4.2	3.2

Table III *The 18 Women (out of 127) Who Stopped Using Oral Contraceptives after 3 Months*

Case	Initial 0	Months after Start	
		1	3
13	1.2	0.8	1.1
24	1.1	0.1	1.4
29	0.5	11.0	7.6
40	1.8	5.9	1.6
51	1.4	3.5	2.9
53	1.4	2.6	8.7
61	0.4	4.5	2.0
64	4.0	4.7	3.8
65	3.1	1.7	1.2
76	3.5	34.1	20.5
88	1.4	0.2	0.6
89	1.0	26.8	17.5
102	1.1	1.3	1.8
108	0.9	1.2	15.3
124	2.3	1.9	1.8
131	3.1	9.6	30.1
136	2.5	13.4	5.5
146	0.6	3.4	1.7
Mean	1.7	7.0	7.0

### Discussion

Treatment with contraceptive steroids places an additional load on the excretory function of the liver (Adlercreutz 1964 Elsallo *et al* 1965 Sherlock 1968) and induces changes in the metabolism of carbohydrates (Wynn and Doar 1966) lipids (Wynn *et al* 1966 Aurell and Cramer 1966) and proteins (Robertson 1967). Many cases of icterus after contraceptive steroids have been reported (*e.g.* Cullberg *et al* 1965 Orellana Alcalde and Domínguez 1966) and it is considered that a relationship exists between peroral contraceptives and icterus (Balnes 1965 Larsen Colm 1965). One of the aims of the present study was to assess the incidence of clinically relevant effects on the liver. The 5 cases with itching and fatigue (2 of which also displayed icterus) all had high S-OCT levels (*cf.* Fig. 4). The symptoms

group C (rise group) was significantly higher than in either of the other two groups.

The present results differ from those reported in many large studies for instance in the United States (Siwaab 1964 Tyler 1964). The explanation for the higher incidence of clinically relevant liver reactions (4 per cent) is partly that this incidence appears to be greater in Scandinavia than in other parts of the world and partly that women who develop secondary effects stop using oral contraceptives and consequently tend to eliminate themselves from the subjects included in large retrospective studies. The relatively high proportion of transient liver reactions is partly due to the prospective nature of the present study with frequent blood tests and partly to the use of S-OCT analysis which is the most sensitive test available for demonstrating liver reactions.

S-OCT analyses appear to be of practical clinical value in two contexts

- 1 In the case of women who call for particular caution during treatment with oral contraceptives e.g. after a recent hepatitis or a suspected syndrome of recurrent cholestatic jaundice of pregnancy (Editorial Brit. Med. J 1967 Sherlock 1968)
- 2 In the case of women who during treatment with oral contraceptives display symptoms suggestive of liver disturbances, e.g. itching.

A normal S-OCT level in these contexts is a strong indication that the oral contraceptives have not elicited liver injury

### SUMMARY

Out of 127 women who used oral contraceptives for at least 3 months, 5 (4 per cent) developed an itch (2 also developed icterus) that disappeared when the medication was stopped all 5 had pathologically elevated serum OCT. Out of 102 women who used oral contraceptives for one year 47 (46 per cent) displayed no or only a slight and transient rise in S-OCT 37 (36 per cent) a pathological but transient rise in S-OCT and 18 (18 per cent) a pathological and successively increasing rise in S-OCT

*et al* 1955 Kyle and Hess 1956) Oral contraceptive steroids are reported to result in an increase in lean body mass and a positive nitrogen balance (Lecocq *et al* 1967) and an increased protein synthesis should ultimately lead to an enhanced breakdown of protein and production of urea. This may be a reason why the women in our study displayed a rise in S-OCT

There is morphological evidence that the synthesis of protein in the liver increases in conjunction with the use of oral contraceptives. Thus an increase in the free ribosomes arranged in rosettelike and spiral formations has been demonstrated by electronmicroscopy of the human liver after the administration of methandrostenolone (Orlandi and Jézéquel 1962) and it has been shown that such findings are associated with increased production of proteins

Some of the present women particularly those in group C (rise group) displayed such a pronounced rise in S-OCT that this can hardly be ascribed solely to a corresponding rise in the production of OCT in the liver. It seems more probable that these pronounced rises in S-OCT reflect changes in the permeability of the mitochondria. Liver biopsies and electronmicroscopic examination performed on women using oral contraceptives have disclosed ultra structural changes in the mitochondria (Orlandi and Jézéquel 1962 Anberg, 1966) and it is to these that OCT are mainly located (Krebs *et al* 1955)

The transient rise in S-OCT displayed by nearly all the women in our study thus seems to be ascribable to increased enzyme synthesis and/or mitochondrial changes. Once the liver has adjusted to the increased load the synthesis and/or the leakage of enzymes decreases and S-OCT is normalized. The present study also disclosed transient rises in S-GOT S-GPT and serum alkaline phosphatase. This is in good agreement with studies by Larsson-Cohn (1967) who reports that in 22 women, the frequency of elevated transaminases in serum was maximum after 1-2 months of therapy and then gradually decreased while the frequency of increased bromsulphalein (BSP) retention reached a maximum after 3 months of therapy and then remained unchanged.

It is interesting to note that the mean initial S-OCT level in

- Notalla, B. *Scand. J. Clin. Lab. Invest.* 12 suppl. 49 1960
- Ordeli R. O. G. *Opuscula Medica* (Stockholm) 1 14 1956
- Orlandi F. and Jézéguel A. M. in *Actuelle Probleme der Hepatologie*  
G. A. Martini ed., p. 41 1952, Georg Thieme Verlag, Stuttgart
- Orrellana-Alcalde J. M. and Dominguez J. P. *Lancet* 2 1278 1966
- Roscherd, H. *Acta Med. Scand. Suppl.* 90 1962
- Roscherd, H. *J. Lab. Clin. Med.* 63 1031 1964
- Robertson G. S. *Lancet* 1 232, 1967
- Ross K. *Scand. J. Clin. Lab. Invest.* 15 suppl. 69 p. 233 1963
- Schwenker R. T. *J. Biol. Chem.* 238 1012, 1953
- Shurtlech S. *Brit. Med. J.* 1 227 1968
- Sweet L. *Brit. Med. J.* 2 755 1954
- Thulin K. E. and Nermmark J. *Brit. Med. J.* 1 584 1956
- Tyler E. T. *Brit. Med. J.* 2 843 1954
- Wroblewski F. and LaDus J. S., *Proc. Soc. Exp. Biol. Med.* 90 210 1955
- Wyse V. and Doer J. V. H. *Lancet* 2 715 1956
- Wyse V. Doer J. V. H. and Mills G. L. *Lancet* 2 720 1956
- Arberg, A. *Acta Obstet. Gynec. Scand* 65 suppl. 9 p. 40 1956

Received on March 28, 1968

The rises in serum OCT are probably partly due to altered permeability as a result of mitochondrial changes and partly to an increased synthesis of OCT in the liver. An analysis of serum OCT seems to be of prognostic value with respect to liver injuries in women who use oral contraceptives.

### Acknowledgements

This study was supported by a grant from Stockholm County Council Stockholm Sweden.

### REFERENCES

- Adlercreutz H. *Nord. Med.* 72 1004 1964  
 Allgén L.-G. *Svenska Läkartidn.* 59 1847 1962  
 Aurell M and Cramér K. *Lancet* 2 1314 1966  
 Baines G F. *Lancet* 1 108 1955  
 Beckman V, Brohult J and Reichard H. *Acta Anaesth. Scand.* 10 33, 1966  
 Brohult J. *Acta Anaesth. Scand.* 11 201 1957  
 Brohult J, Nilsson U and Olsson K. E. *Acta Orthop. Scand.*, in press 1968  
 Brohult J. In manuscript 1968  
 Brohult J and Westgren A. *Lancet* 2 1344 1965  
 Buch I and Buch H. *Acta Med. Scand.* 101 211 1939  
 Cullberg, G, Lundström R. and Stenram U. *Brit. Med. J.* 1 695 1965  
 Dixon W J and Massey F J. *Introduction to statistical analysis* New York, 1957  
 Editorial. *Brit. Med. J.* 4 499 1957  
 Eisalo A, Järvinen P A. and Luukkainen T. *Brit. Med. J.* 2 426 1964  
 Jendrassek L and Gróf P. *Biochem. Z.* 297 81 1938  
 Karmen A. *J. Clin. Invest.* 34 131 1955  
 Knutsson F, Rybo G and Anberg, A. *Acta Obstet. Gynec. Scand.* 44 325 1965  
 Krebs H A, Eggleston L V and Knipper V A. *Biochem. J.* 59 185 1955  
 Kyle L H and Hess W L. *J. Lab. Clin. Med.* 47 276 1956  
 Landau R L, Bergenstal D M, Lugibuhl K and Kascht M E. *J. Clin. Endocr. Met.* 15 1194 1955  
 Larsson-Cohn U. *Brit. Med. J.* 1 1414 1965  
 Larsson-Cohn U. *Acta Obstet. Gynec. Scand.* 45 499 1966  
 Larsson-Cohn U. *Amer. J. Obstet. Gynec.* 93 188 1967  
 Lecocq F R, Bradley E M and Goldzieher J W. *Amer. J. Obstet. Gynec.* 99 374 1967  
 MacLagan N F. *Brit. J. Exp. Path.* 25 234 1944

Table I. Distribution of Patients According to Age and Parity

Age	Par 0	Para 1-2	Par 3-4	Para 5-6
20	3	1		
20-29	2	10	5	
30-39		5	3	1
>39	1	10	2	1
Totals	11	26	10	2

One or two days before the operation of conization the patients were subjected to hysterosalpingography essentially in the way described by Asphund (1952) in his investigation of the radiological appearance of the cervix and isthmus. In the present series to avoid masking of the cervix by the cone of the instrument the cone was usually cut down. The contrast medium used was viscous perijodal H.

The radiological examination was repeated about four weeks after operation and again 6 to 12 months later using the same contrast medium and the same projections and, when possible on the same day of the menstrual cycle as the primary examination. The pre- and postoperative films were then compared to assess the calibre of the cervix (approximately estimated as small, average, large) the diameter of the internal os (mm in frontal plane) and the pattern of the cervical mucosa (well defined plicae palmatae plicae seen only as a serrated line and plicae not visible) Notes were also made of any abnormalities such as irregular pattern of the mucosal relief pockets, filling of glandular ducts, synechiae and polyps.

Further specimens for histological examination were available from seven women reoperated upon because of a suspected local recurrence within 3 months (1 patient) 7 months (2 patients) 11-13 months (3 patients) and 24 months (1 patient) of the primary operation.

When patients had become pregnant after the operation, and until 1967 details of their histories during pregnancy and the puerperium were obtained from the hospital records.

## THE UTERINE CERVIX BEFORE AND AFTER COLD KNIFE CONIZATION

BY

STIG KULLANDER AND LENNART WEHLIN

Cold knife conization is widely practised in the treatment of cytologically suspected cancer of the uterine cervix stage 0. It is stated that this procedure does not interfere with reproduction but it is not known whether the cervix is afterwards anatomically and physiologically normal. Some investigators believe that cervical disorders with infertility, abortion and premature births, are more common after conization. Late symptoms resulting from cervical stenosis have been described but not properly investigated. With the increasing frequency of cytological examination of cervical smears and the increasing number of young women subjected to conization of the uterine cervix it is important to ascertain whether any permanent anatomical or physiological sequelae occur after the operation. This problem has been studied on the basis of the patient's history, hysterosalpingography and histological examinations.

### *Material and Methods*

The clinical material consisted of 44 women aged 16 to 49 years who were subjected to cold knife conization in 1962 or 1963. The ages and parities of the patients are shown in Table I. In 10 of the specimens obtained at operation the histological changes in the epithelium were less than that of fully developed carcinoma *in situ*.





Fig 1 A 37 year old Para II in w  
 cancer sirs had been radically e  
 pated Normal pregnancy one year  
 operation Cervix before ( ) 3 w  
 (b) and 10 months after ( ) conu  
 Normal findings

Table II. Preoperative Roentgen Appearance of Cervix. (Italics give number of cases in which cancer *in situ* could not be proven histologically)

Phase of Menstrual Cycle	Width of Cervix		Internal Os (mm)	Mucosal Relief			Abnormal Findings
	Small	Average		Large	No Visible Plicae	Slight Plication	
Proliferative		29	1-9 (mean 4.9)	13+2	10+2	1+1	0
Secretory		13	2-8 (mean 4.3)	4+3	5	1	0

## Results

### Radiological findings

Table II gives the preoperative appearance of the cervix in 42 menstruating women grouped according to the menstrual phase at the time of examination (judged from the histological appearance of the endometrium at operation) and according to whether the histological diagnosis was cancer *in situ* or only slight atypical epithelial proliferation. The table shows that the width of the cervix the diameter of the internal os and the mucosal pattern were normal before operation. Hence the radiological examination did not allow a diagnosis of carcinoma *in situ* not even when the growth extended down into the glands. In 11 cases histological examination of serial section showed some down growth of intraepithelial cancer in the cervical glands. In this group also roentgen examination showed no evidence of the lesion.

The appearance of the cervix 6 months to 1 year after the operation is shown in Table III. The cervix healed with complete restoration of the original form and of the mucosal relief. No widening capable of causing cervical insufficiency was observed. The women with the widest internal os after operation (10 mm on 12th day of cycle) later gave birth to a healthy boy weighing 2660 g. Neither pregnancy nor delivery was complicated.

The return of the radiological appearances to normal was seen very soon after the operation. When the patients were examined as early as 3-4 weeks after the operation the appearances were the same except that there were occasionally pockets in the cervical wall which were no longer demonstrable at later ex-

Table III. Radiological Appearance of Cervix 6 Months to 1 Year after Conization. (In give number of cases where cancer *in situ* could not be proven histologically)

Day of Cycle	Width of Cervix		Internal Os (mm)	Mucosal Relief			Abnor. Flodin
	Small	Average Large		No Visible Plicae	Slight Plication	Well Defined Plicae	
0-12	28		2-10 (mean 4.6)	12+3	8+2	3	0
>12	14		3-8 (mean 5.3)	6-3	2+1	0+1	0

*Postoperative functional state of cervix*

The operation did not appear to interfere with later function of the cervix. Thus, of 32 of the women in whom pregnancy might be expected, 13 (41 per cent) became pregnant. Of the remaining patients, 3 had radiologically diagnosed bilateral hydrosalpinges before the operation, 5 used contraceptive pills after the operation, 1 had a hysterectomy because of a recurrence of carcinoma *in situ* and 3 were more than 43 years old. These 13 women in whom pregnancy was possible had 17 pregnancies within the first 4 years after the operation. Two of the pregnancies ended in spontaneous abortion. None of the children were born before term and none of the pregnancies were complicated.

The functional state of the cervix after conization has received little consideration. Ellasson *et al.* (1964) reported that abortion occurred in 26 per cent of 31 pregnancies in women who had been subjected to conization. Of 23 pregnancies in 56 women who were below 35 years and who had previously undergone conization because of cancer *in situ* Boyd *et al.* (1963) found that 22 per cent terminated in abortion. Geen (1966) reported 30 pregnancies among 231 women who had undergone conization because of cancer *in situ* and concluded that the operation did not impair fertility as 43 per cent of those women in whom conception could be expected became pregnant. In his series the abortion frequency was 18.4 per cent. He thought that abortion, premature delivery and cervical dystocia were more common after conization.



2a



2b

Fig. 2. A 16 year old 0-para with cancer *in situ*. Normal pregnancy 4 years after operation. Cervix before (a) and 6 weeks after (b) conization. Normal findings.

aminations. These pockets had presumably been caused by inadvertent cuts in cervical muscle by the tip of the surgeon's knife. That healing was rapid and good is illustrated in Figs 1-3. A "cervical pocket" is shown in Fig. 4.

### *Histological findings*

All of the cones removed at repeat operation showed regeneration of the lining epithelium and of the crypts and glandular ducts. In four cases well developed plicae palmatae were seen in transverse sections of the cervix. In one case no plicae could be seen with certainty and in the remaining 2 cases the sections would not allow evaluation in this respect.

For help with the histological examinations we thank As. P. of Berge, Dept. of Pathol. (Head, Prof. F. Linell), Malmö.



Fig 4 A 27 year old Para II Cervix after operation. A cervical pocket, 10 mm by 4 mm, with its base downward (↓)

### SUMMARY

Forty four women aged 16-49 years were operated upon in 1962-1963 with cold-knife conization without subsequent suturing, because of suspected cancer of the uterine cervix stage 0. The patients were subjected to hysterosalpingography before, and at 1 and at 6 to 12 months after the operation.

The cervix soon recovered its preoperative roentgen appearances. There was no widening of the cervical canal. Formation of new glands was shown histologically.

By 1967 13 of the 32 women who were capable of conceiving had a total of 17 pregnancies (conception rate 41 per cent). Only 2 of these pregnancies ended in spontaneous abortion. All the pregnancies were normal and there were no premature births.



3a



3b



3c

Fig 3 A 22 year old 0-para with cancer *sit*. Cervix before (a) 8 weeks after (b) and 6 months after (c) conization. Normal findings



Fig. 4. A 27 year old Par II. Cervix after operation. A cervical pocket, 10 mm by 4 mm, with its base downward. (1)

### SUMMARY

Forty four women aged 16-49 years were operated upon in 1962-1963 with cold-knife conization without subsequent suturing, because of suspected cancer of the uterine cervix stage 0. The patients were subjected to hysterosalpingography before and at 1 and at 6 to 12 months after the operation.

The cervix soon recovered its preoperative roentgen appearance. There was no widening of the cervical canal. Formation of new glands was shown histologically.

By 1967 13 of the 32 women who were capable of conceiving had a total of 17 pregnancies (conception rate 41 per cent). Only 2 of these pregnancies ended in spontaneous abortion. All the pregnancies were normal and there were no premature births.



Fig. 3 A 22 year old 0-para with cancer in situ. Cervix before (a) 6 weeks after (b) and 6 months after (c) conization. Normal findings



## TORSION OF THE PREGNANT HUMAN UTERUS

BY

MAGNAR KNUT ULSTEIN

Torsion of the pregnant human uterus is a very rare condition. Only 129 cases have been described. Towards the end of pregnancy a slight rotation of the uterus may occur normally. In 60 per cent of cases this physiological rotation occurs in a clockwise direction and more than 30 degrees is considered pathological (Nowoselski and Henderson 1960). Further rotation will give rise to symptoms depending on the degree of torsion, the stage of pregnancy and the speed at which the torsion itself develops. Two cases have been described with torsion of 540 degrees (Olwe 1910 and Schnidler 1919) and one case with as much as 720 degrees (Syme 1906).

### Case report

The patient was 31 years old, admitted Feb. 11, 1963. Forceps delivery in 1949. The infant was deformed and died after 4 days. Two years later she had normal delivery. The third pregnancy, the one under consideration, was normal until last weeks before term. She then developed mild toxæmia. Transverse lie had been present for several weeks. Correction to head presentation was easily done, but as soon as the patient lay on her back the foetus slipped back to transverse lie. She had no abdominal pains or other striking symptoms. Vaginal examination showed closed cervix, an unusual finding in primipara III at term. It was not possible to reach the membranes or the presenting part when the foetus was pressed down into the pelvic inlet. The foetus seemed to be full term. Radiography showed transverse lie and normal pelvis. Three days after the expected date of delivery she was operated on. On opening the abdomen the uterus presented once rather unusual picture. The peritoneum over the lower uterine segment was wrinkled. The fibrous end of one tube was visible deep on the left side. There were some tense strands over the lower part of the cervix. The round ligaments

## REFERENCES

- Asplund J* Acta radiol. Suppl 91 1972  
*Boyd J R. Rayle D Fidler H A. and Boyes D A.* Am. J Obstet. Gynec.  
85 322 1963  
*Eliasson G Lundgren N and Norden J G* Sv Läk Tidsn. 61 1974 1934  
*Green J H* J Obstet. Gynaec. Brit. Cwlth 73 897 1966

Received on March 26 1968

the 9th month of pregnancy. In 2 cases of uterine myoma the stage of pregnancy was not mentioned. In cases with no pathological findings there was an increasing incidence towards term with 1 case before the 5th month of pregnancy, 3 in the 5th-6th months, 7 in the 7th-8th months and 11 in the 9th month of pregnancy. When abnormal foetal presentation was found, torsion occurred in the last 2 months of pregnancy. In cases of uterine anomalies and of adhesions, torsion was most common in the 2nd third of pregnancy.

### *Symptoms*

The most common symptom was abdominal pain, found in 107 cases. Only 6 patients were symptom-free. When the patient had labour pains, partus prolongatus was an important finding (23 cases). Symptoms of shock were found in 31 patients, vaginal bleeding in 10 patients, urinary tract symptoms in 18 and intestinal symptoms in 34 patients. In most cases the symptoms developed acutely indicating that torsion occurred as a sudden catastrophe. Exceptions to this were the 6 symptom free cases in which Caesarean section was carried out for other reasons. In 28 cases torsion developed in connection with partus. Sudden body movements of the mother have been suggested as predisposing factors (Björkenheim, 1965).

### *Diagnosis*

No special symptoms or signs can lead to diagnosis. Torsion was usually a surprising finding at operation in cases presenting urgent reasons for laparotomy without an exact diagnosis having been made. In 7 cases diagnosis was not made until autopsy. On clinical examination the cervix is closed. The presenting part of the foetus cannot be reached. The round ligament may be palpated as a tender strand on the anterior surface of the uterus. Differential diagnosis early in pregnancy can include all acute abdominal conditions. Later in pregnancy the most important differential diagnosis are rupture of the uterus, ablatio placentae or acute hydramnion.

were not visible. The uterus was rotated 180 degrees, and the right adnexa were pulled across to the left side. The left tube and ovary were concealed by the uterus on the right side. The uterus was easily manipulated to normal position. Nothing pathological was found in regard to the uterus or adnexa. Caesarean section was carried out in the usual way with a low transverse incision and a living male child, weighing 3870 g, was extracted breech first. There were no postoperative complications.

Torsion of the nonpregnant uterus has been described by *Times* in 1861 and later by *Virchow* in 1863. Both instances were noted in combination with uterine fibroids at autopsy. In 1876 *Labbe* published the first case of torsion of the pregnant human uterus. More recently there have been several reports of individual cases and a few surveys. The total number of described cases is now 130 including the present one. Seventeen cases in the literature were insufficiently detailed to be considered in the following brief survey.

### Aetiology

In 1904 *Barro* +1 maintained "No tumour, no torsion." *Robinson* and *Duvall* (1931) modified this to "No uterine abnormality, no torsion." *Nesbitt* and *Corner* (1956) state "No pelvic pathology, torsion unlikely." However it appears that torsion can occur without extant pathological changes in the uterus, adnexa or pelvis.

Uterine myoma were present in 36 cases, uterine anomalies in 16, adhesions in 14, abnormal presentation of the foetus in 8, pelvic deformity in 3, scarred cervix in 2, placenta praevia in 3, ovarian tumour in 7, and interstitial pregnancy in 2 cases. However the group with no pathological changes was also large with 22 cases in all. In the 8 cases with abnormal foetal presentation the uterus, adnexa and pelvis were normal.

The parity was unknown in 4 cases, 46 were primigravida and 63 were plurigravida. The rotation was clockwise in 66 cases, anti-clockwise in 39, and unknown in 8 cases. The torsion was most common in the cervical region.

In cases of uterine myoma torsion occurred most frequently early in pregnancy, 19 cases before the 5th month of pregnancy, 6 cases in the 5th-6th months, 3 in the 7th-8th months, and 7 in

the 9th month of pregnancy. In 2 cases of uterine myoma the stage of pregnancy was not mentioned. In cases with no pathological findings there was an increasing incidence towards term with 1 case before the 5th month of pregnancy, 3 in the 5th-6th months, 7 in the 7th-8th months and 11 in the 9th month of pregnancy. When abnormal foetal presentation was found, torsion occurred in the last 2 months of pregnancy. In cases of uterine anomalies and of adhesions torsion was most common in the 2nd third of pregnancy.

### Symptoms

The most common symptom was abdominal pain, found in 107 cases. Only 11 patients were symptom-free. When the patient had labour pains, partus prolongatus was an important finding (23 cases). Symptoms of shock were found in 31 patients, vaginal bleeding in 10 patients, urinary tract symptoms in 18 and intestinal symptoms in 34 patients. In most cases the symptoms developed acutely indicating that torsion occurred as a sudden catastrophe. Exceptions to this were the 6 symptom free cases in which Caesarean section was carried out for other reasons. In 28 cases torsion developed in connection with partus. Sudden body movements of the mother have been suggested as predisposing factors (Björkenheim, 1965).

### Diagnosis

No special symptoms or signs can lead to diagnosis. Torsion was usually a surprising finding at operation in cases presenting urgent reasons for laparotomy without an exact diagnosis having been made. In 7 cases diagnosis was not made until autopsy. On clinical examination the cervix is closed. The presenting part of the foetus cannot be reached. The round ligament may be palpated as a tender strand on the anterior surface of the uterus. Differential diagnosis early in pregnancy can include all acute abdominal conditions. Later in pregnancy the most important differential diagnosis are rupture of the uterus, ablatio placentae, or acute hydramnion.

### *Treatment*

In the majority of cases treatment has been laparotomy. Nine patients were not operated on. In 47 cases Caesarean section was carried out, in 26 hysterectomy, in 13 myomectomy, in 6 ovariectomy and in 12 cases laparotomy and detorsion of the uterus. The treatment to be recommended is laparotomy in time. Early in pregnancy detorsion and if possible removal of the cause should be carried out. Where the foetus is viable Caesarean section should be performed.

### *Prognosis*

Both foetal and maternal mortality have been high. The respective figures being 43 per cent and 15 per cent. When the torsion occurred after the 28th week of pregnancy the foetal mortality was 37 per cent. In 15 cases there was both foetal and maternal death. The maternal mortality was highest when torsion occurred in the later stages of pregnancy. Nesbitt and Corner (1956) point out that both foetal and maternal mortality were highest in the cases first published. The same authors showed that maternal mortality increases with increasing degree of torsion. Caesarean section was performed in 34 of the cases with torsion of the uterus in the last month of pregnancy. Of these 3 mothers and 4 infants died. The 5 untreated cases in the same month of pregnancy all died, both mothers and infants.

### *Discussion*

Whether transverse lie predisposes to torsion or vice versa is conjectural. It may be pointed out that transverse lie does lead to asymmetry of the uterus. Irregularity in uterine form seems to be an important aetiological factor (Imrie 1966, Mitchell and Garrett, 1960). In our case transverse lie had been present for several weeks before admission. It can be noted that all of the published symptom free cases were at term. The diagnosis was made by chance at Caesarean section carried out for other reasons. In 2 cases there was transverse lie, in 2 adhesions, in 1 pelvic deformity and in 1 case uterine myoma.

Although the condition is very uncommon, one should be aware of it, for if the uterus is opened before detorsion is carried out, massive bleeding may occur

### SUMMARY

A case is reported with 180 degrees of torsion of the uterus in a full term pregnancy. There were no symptoms. The diagnosis was made by chance at Caesarean section carried out because of transverse lie.

### REFERENCES

- Berrozti, J. Cited by Nesbitt and Corner  
Björkman, E. A. *Acta obstet. gynec. scandinav.* 44 18 1965  
Isure, A. H. *J. Obstet. Gynaec. Brit. Comm.* 73 1022, 1966  
Labbe, L. Cited by Nowinskiak and Henderson  
Mitchell, P. R. and Garrett, W. J. *J. Obstet. Gynaec. Brit. Emp.* 67 654, 1960  
Nesbitt, R. and Corner, G. *Obstet. Gynaec. Survey* 11 311 1956  
Nowinskiak, P. and Henderson, H. *Amer. J. Obstet. Gynec.* 80 272, 1960  
Olav, J. *Monatsschr. f. Geburtsh. u. Gynäk.* 32 53, 1910  
Robinson, A. and Dunell, H. *J. Obstet. Gynaec. Brit. Emp.* 38 55 1931  
Schmüller, R. *Monatsschr. f. Geburtsh. u. Gynäk.* 50 409 1919  
Syme, G. *Lancet* 1 518, 1908  
Turner, H. Cited by Nowinskiak and Henderson  
Virchow, R. *Die krankhaften Geschwülste*, III, 1863

Received on April 10 1968

Author's present address

Östr. Sjukhuset,

K. smoksluken

Göteborg S

## THE EFFECT OF HISTAMINASE INHIBITION ON THE CONCENTRATION AND DISTRIBUTION OF $^{14}\text{C}$ HISTAMINE IN BLOOD DURING PREGNANCY

BY

ÅKE TÖRNQVIST

The histaminolytic activity of blood plasma is considerably increased during human pregnancy (Marcou *et al* 1938 Ahlinark 1944) Lindberg (1963) showed that the concentration of  $^{14}\text{C}$  histamine in arterial blood of pregnant women was approximately 50 per cent lower than that of non pregnant women during intravenous infusions of equal doses of isotope labelled histamine Using the same technique Lindberg and Törnqvist (1966) studied the blood concentration of histamine in pregnant women after inhibition of the enzyme histaminase by means of intramuscular injection of aminoguanidine. The pattern of the histamine metabolites *i.e.* the absence of imidazoleacetic acid in the blood, indicated that the inhibitory effect of the aminoguanidine was complete Lindberg and Törnqvist (1966) also found that that the arterial blood concentration of  $^{14}\text{C}$  histamine in pregnant women was higher after histaminase inhibition as compared with the conditions when the enzyme was left intact (Lindberg, 1963)

In view of these results it was surprising that previous investigators (Janowitz and Grossman 1949 Wicksell 1949 Kulander 1952 Clark and Tankel 1954) did not find any difference in the biological effects of exogenous histamine between pregnant and non pregnant women

To elucidate this problem further the effects of infused histamine on the heart rate and on the forearm blood flow were studied in pregnant and non pregnant women before and after inhibition of histaminase (Törnqvist 1968 a and b) In these



Investigations Törnqvist found significantly higher effects of the infused histamine on the heart rate and on the forearm blood flow in the non-pregnant than in the pregnant women. The responses to histamine were also significantly increased in the pregnant group after the inhibition of histaminase.

The aim of the present investigation was to study the concentration of  $^{14}\text{C}$  histamine in arterial blood after infusion of isotope labelled histamine under similar experimental conditions to those which were used in the studies of the biological effects of exogenous histamine (Törnqvist 1968 a and b). In investigations by Lindell and Wikke (1961) and by Lindell and Westling (1962) most of the  $^{14}\text{C}$  histamine recovered from the blood, after intravenous infusion of isotope labelled histamine, was found in the plasma. In the present investigation the concentration of  $^{14}\text{C}$  histamine therefore has been studied in the plasma and the blood cell fraction as well as in total blood. The disappearance of  $^{14}\text{C}$  histamine in total blood *in vitro* was also investigated.

### Materials

Fifteen physically healthy pregnant women were studied. They were admitted to the clinic for legal abortion and sterilization and were in the 17th to the 22nd week of pregnancy counting from the first day of the last menstrual period. Information regarding these pregnant women is given in Table I.

The infused isotope labelled histamine was  $^{14}\text{C}$  histamine (histamine dihydrochloride labelled in the 2 position of the imidazole ring) obtained from the Radiochemical Centre, Amersham, England. The non-isotopic carrier was histamine dihydrochloride obtained from E. Merck A. G. and the aminoguanidine used for inhibition of the enzyme histaminase was aminoguanidine sulphate (Eastman Organical Chemicals).

### Methods

In an attempt to avoid uncomfortable histamine effects all subjects received 50 mg promethazine (Lergigan, Recip) intramuscularly half an hour before the beginning of the investiga-

Table 1. Description of Cases and Infusion Rates of  $^{14}\text{C}$  Histamine

Case	Age in Years	Duration of Pregnancy in Weeks	Body Weight Kg	Infusion Rate of $^{14}\text{C}$ Histamine in cpm	Infusion Rate of $^{14}\text{C}$ Histamine in cpm/kg of Body Weight
1	35	19	69	39 000	565
2	38	20	60	35,000	580
3	31	18	86	41,000	480
4	34	18	91	23 000	250
5	40	22	56	23,000	410
6	40	19	68	45,000	660
7	34	17	59	44,000	750
8	38	17	69	42,000	610
9	31	19	67	22,000	330
10	41	18	71	39 000	550
11	29	20	71	41,000	80
12	38	17	61	-	-
13	37	19	78	-	-
14	34	19	67	-	-
15	42	20	39	-	-

Infusion rate of histamine in counts per minute (cpm)

tion. All investigations were done in the morning with the subjects fasting

*Infusion of histamine* Isotope labelled histamine was infused intravenously into the right arm by means of a motor-driven syringe. The histamine was dissolved in 0.9 per cent saline. Infusion rates of 12 and 21  $\mu\text{g}$  of histamine base per minute were used. Infusion periods of 6 minutes were chosen to correspond with the experimental conditions in previous investigations on some biological effects of exogenous histamine (Törnqvist 1968 a and b). All subjects received two infusions of histamine with 30 minutes' interval. In the histamine free intervals physiological saline solution was infused, at a low speed to prevent clotting in the needle and the vein.

*Inhibition of histaminase* The histaminase was inhibited by intramuscular injection of 0.2 mg of aminoguanidine sulphate per kg of body weight immediately after the first infusion of histamine was finished. The injected solution contained 10 mg of

aminoguanidine sulphate per ml of sterilized water. The histaminase level in plasma was studied in 6 pregnant women before and after the injection of aminoguanidine. A biological testing method described by Ahlmark (1944) showed that the plasma histaminase activity half an hour after the injection of the aminoguanidine in all cases was decreased almost to a non-pregnant value. This and other investigations (Brody Lindberg and Törnqvist unpublished) showed that 30 minutes were sufficient for an almost complete inhibition of the histaminase activity in human plasma.

*Sampling of blood.* Immediately before stopping the infusion of histamine blood was withdrawn from a polythene catheter which had been inserted previously in the left brachial artery under local anaesthesia as described by Bernius Carlsten Holmgren and Seidinger (1954). Ten ml samples were usually taken for analyses. Blood samples were always obtained with heparine treated syringes. To separate the blood cells from the plasma the blood was centrifugated for 8 minutes at a rate of 6000 rev/minute at room temperature (the diameter of the centrifuge head was about 24 cm).

*Assay of histamine.* The plasma and the blood cell fraction were transferred 8 minutes after sampling to previously weighed glass tubes with known volumes of non-isotopic carrier. The distribution of the infused histamine was studied in 11 cases. In 6 of these the concentration of isotope labelled histamine in total blood was also estimated. In these 6 cases one part of the blood was mixed with carrier immediately after sampling. Another part of the same blood sample was mixed with carrier 8 minutes after sampling. In this way it was possible to study the histaminolysis *in vitro* at room temperature during the time necessary to separate the plasma and the blood cell fraction. The concentration of histamine was measured by Schayer's isotope dilution technique (Schayer and Cooper 1956 Lindell and Schayer 1958). The blood samples were mixed with carrier equivalent to 206 mg of the corresponding picrate. Trichloroacetic acid was added to give a final concentration of about 5 per cent, thus precipitating the proteins. The samples were filtered and the acid removed from the filtrate with ether. The filtrate was saturated with

anhydrous  $\text{Na}_2\text{SO}_4$  and made strongly alkaline with  $\text{NaOH}$ . The histamine was extracted with *n* butanol and the butanol fraction was extracted with  $\text{HCl}$ . The  $\text{HCl}$  extract was evaporated to dryness. The histamine-dihydrochloride so obtained was dissolved in water and picric acid. The histamine-dipicrate was converted to histamine-dihydrochloride by passage through a Dowex anion exchange column and was made to react with *p*-iodophenyl sulphonyl chloride (pipsylchloride). Pipsylhistamine was recrystallized and treated with activated charcoal several times until three successive crystallizations showed fairly constant radioactivity. The radioactivity was measured at infinite thickness in a flow counter operated with methane. At least 1000 counts were taken after every recrystallization. The constant value for each sample was corrected for background radioactivity and expressed as counts per minute per ml of blood (cpm/ml). One  $\mu\text{g}$  of the  $^{14}\text{C}$  histamine base used in this study gave about 2000 cpm above background.

### Results

In 11 cases (cases 1–11) the concentration of  $^{14}\text{C}$  histamine was estimated in the plasma and in the blood cell fraction before and after inhibition of histaminase. The results are given in Table II. The concentration of isotope labelled histamine in cpm/ml increased in both fractions after inhibition of histaminase.

In 6 cases (cases 6–11) the investigation was extended to estimate the concentration of isotope labelled histamine in total blood. These results are also given in Table II. In most cases the concentration of histamine in total blood was increased after inhibition of the histaminase. This increase however was much smaller in those blood samples which were immediately mixed with non isotopic carrier (total blood I) than in the samples which were mixed with carrier 8 minutes after sampling (total blood II). Before inhibition of histaminase there was a considerable disappearance of histamine in the blood samples which were mixed with carrier 8 minutes after sampling. After inhibition of histaminase however there was no corresponding histaminolysis. The influence of this histaminolysis *in vitro* on the

Table II Concentration of  $^{14}\text{C}$  Histamine in Plasma, Blood Cell Fraction and Total Blood in Counts per Minute per ml (cpm/ml) Donor &  $t$  various fractions of Labelled Histamine

Case	Infusion Rate of Histamine $\mu\text{g}/\text{min}$	Before Inhibition of Histaminase				After Inhibition of Histaminase			
		Plasma	Blood Cell Fraction	Total Blood I	Total Blood II	Plasma	Blood Cell Fraction	Total Blood I	Total Blood II
1	21	29,000	47	2.4		120	4.4	-	-
2	21	35,000	74	2.5		11.5	5.1	-	-
3	21	41,000	5.9	2.5		13.9	6.0	-	-
4	12	23,000	3.9	1.9		6.7	3.4	-	-
5	12	23,000	2.4	1.4		8.9	3.7	11.7	11.9(11.9)
6	21	45,000	21	1.7	7.0	15.8	6.8	9.1	12.9(13.5)
7	21	44,000	70	4	9.2	17.5	7.1	11.0	10.4(11.0)
8	21	42,000	17	1.5	5.9	14.6	6.4	4.9	4.8(4.9)
9	12	22,000	26	1.4	4.0	6.2	3.1	11.6	12.7(16.2)
10	21	9,000	71	3.0	8.7	18.7	12.5	8.3	12.3(11.5)
11	21	41,000	6.3	3.3	6.0	15.4	5.6		

Total blood I represents the blood fraction which was immediately mixed with non-leucocyte carrier and total blood II the fraction which was mixed with carrier & sediments after sampling.

The figures in brackets indicate the histamine concentration in total blood which was calculated from the corresponding values in plasma and blood cell fraction.

anhydrous  $\text{Na}_2\text{SO}_4$  and made strongly alkaline with  $\text{NaOH}$ . The histamine was extracted with *n* butanol and the butanol fraction was extracted with  $\text{HCl}$ . The  $\text{HCl}$  extract was evaporated to dryness. The histamine-dihydrochloride so obtained was dissolved in water and picric acid. The histamine-dipicrate was converted to histamine-dihydrochloride by passage through a Dowex anion exchange column and was made to react with *p*-iodophenyl sulphonyl chloride (pipsylchloride). Pipsylhistamine was recrystallized and treated with activated charcoal several times until three successive crystallizations showed fairly constant radioactivity. The radioactivity was measured at infinite thickness in a flow counter operated with methane. At least 1000 counts were taken after every recrystallization. The constant value for each sample was corrected for background radioactivity and expressed as counts per minute per ml of blood (cpm/ml). One  $\mu\text{g}$  of the  $^{14}\text{C}$  histamine base used in this study gave about 2000 cpm above background.

### Results

In 11 cases (cases 1-11) the concentration of  $^{14}\text{C}$  histamine was estimated in the plasma and in the blood cell fraction before and after inhibition of histaminase. The results are given in Table II. The concentration of isotope labelled histamine in cpm/ml increased in both fractions after inhibition of histaminase.

In 6 cases (cases 6-11) the investigation was extended to estimate the concentration of isotope labelled histamine in total blood. These results are also given in Table II. In most cases the concentration of histamine in total blood was increased after inhibition of the histaminase. This increase however was much smaller in those blood samples which were immediately mixed with non isotopic carrier (total blood I) than in the samples which were mixed with carrier 8 minutes after sampling (total blood II). Before inhibition of histaminase there was a considerable disappearance of histamine in the blood samples which were mixed with carrier 8 minutes after sampling. After inhibition of histaminase however there was no corresponding histaminolysis. The influence of this histaminolysis *in vitro* on the

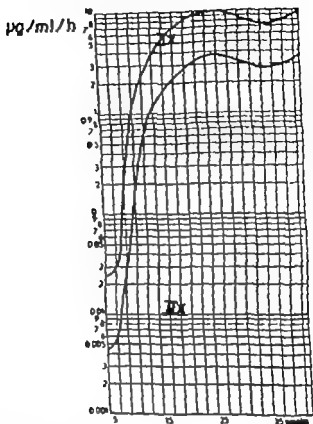


Fig. 1 Histaminolytic activity in blood plasma of pregnant women before (Ix) and after (Ii) inhibition of histaminase by means of intramuscular injection of aminoguanidine

Abscissa: weeks of pregnancy calculated from the first day of the last menstrual period

Ordinate: histaminolytic activity expressed in  $\mu$ g of inactivated histamine per ml of plasma and per hour

plasma was measured, before and after inhibition of histaminase *in vivo* using a biological technique described by Ahlmark (1944)

The results were all similar and showed a marked reduction of the histaminolytic activity after the injection of aminoguanidine. Fig. 1 shows one of these studies.

Table III *Distribution of  $^{14}\text{C}$  Histamine Between Plasma and Blood Cell Fraction in Percentage of Histamine Recovered During Infusion of Labelled Histamine*

Case	Before Inhibition of Histaminase		After Inhibition of Histaminase	
	In Plasma	In Blood Cell Fraction	In Plasma	In Blood Cell Fraction
1	76	24	82	18
2	80	20	74	25
3	72	28	72	28
4	65	35	76	24
5	70	30	78	22
6	62	38	75	25
7	72	28	79	21
8	57	43	74	26
9	71	29	71	29
10	76	24	68	32
11	76	24	81	19

concentrations of histamine in plasma and blood cell fraction in the present investigation will be discussed later

It is possible to calculate the concentration of histamine in that total blood fraction which was mixed with carrier 8 minutes after sampling from the ratio

$$\frac{\text{cpm (plasma)} + \text{cpm (blood cell fraction)}}{\text{ml (plasma)} + \text{ml (blood cell fraction)}}$$

These values are given within brackets in Table II. In most cases the calculated and the directly measured concentrations of histamine correspond well. In case 10 however the directly measured value is much higher than the calculated one. In this case there was good reason to believe that this single sample was contaminated with radioactive material in the laboratory.

The distribution of the recovered isotope labelled histamine between plasma and blood cell fraction is given in Table III. About 1/3–1/5 of the recovered histamine was found in the blood cell fraction both before and after inhibition of histaminase.

In 6 cases (cases 10–15) the histaminolytic activity of the



composition continues outside the organism and that histaminolysis *in vitro* is much greater in the blood of pregnant than in that of non-pregnant women. Lindberg (1963) also showed that this type of histaminolysis can be almost completely inhibited if aminoguanidine is added to the blood immediately after sampling. A detailed analysis of the quantitative importance of histaminolysis *in vitro* in measuring plasma histamine concentrations has not been made in the present investigation. Such an analysis is taking place where aminoguanidine is added to the blood immediately after sampling and before the separation of plasma and blood cells. It has not yet been finished as very few patients have been available.

Histaminolysis *in vitro* in total blood was studied in those cases where part of the blood was mixed with carrier immediately after sampling and part was left in the syringe at room temperature for 8 minutes before carrier was added. Before the injection of aminoguanidine the histaminolysis during this time was considerable but it was almost abolished after the inhibition of histaminase. The degree of *in vitro* histaminolysis also showed considerable variation among the cases in the present investigation.

In an investigation by Lindell and Visker (1961) it was shown that the concentration of histamine during the infusion of histamine to dogs, rose rapidly in plasma but more slowly in the blood cell fraction. When the infusion of histamine to these dogs was stopped, the histamine concentration fell more rapidly in the plasma than in the blood cell fraction. The fact that histaminolysis is not uniform in plasma and blood cells increases the difficulty of evaluating the concentrations of histamine in the present investigation.

A comparison of the histamine concentrations, before and after inhibition of histaminase in the total blood samples which were immediately analysed is less subject to error since the effects of the histaminolysis *in vitro* are probably small. In most cases there was an increase in the total blood concentrations of histamine after the enzyme inhibition. That the concentration in at least one of the cases was not increased at all, may possibly be due to a compensatory increase in the methylation of histamine (Lindberg and Törnqvist 1966).

### Discussion

The infusion of histamine has well known pharmacological effects in man especially on the circulatory system. Earlier investigators in this field were unable to show any measurable increase in histamine concentration in total blood (Rose 1940) and in plasma (Emmelin *et al* 1941 Adam *et al* 1950 and 1954) during intravenous infusion, even if the infused histamine produced marked biological effects. In investigations in cat (Emmelin *et al* 1941) and in man (Adam 1954) biological indicators were considered to be more sensitive than current methods for histamine estimation in blood for detecting an increase in the concentration of free histamine in total blood and plasma. With more refined methods for estimation of the histamine content in total blood and plasma however Adam *et al* (1957) demonstrated that the histamine concentration in plasma was increased during infusion of histamine. With the aid of Schayer's isotope dilution technique labelled histamine could be measured accurately (Lindell and Schayer 1958) and it was possible to correlate the biological effects with the actual concentration of histamine in the blood during histamine infusion (Lindell and Westling, 1962) Lindell and Westling (1962) showed that increase in the pulse rate and flushing of the face were found even if the increase in concentration of histamine in the plasma was very small.

The aim of the present investigation was to study the concentration of  $^{14}\text{C}$  histamine in total blood plasma and blood cell fraction during an infusion of isotope labelled histamine under similar experimental conditions to those which were used in earlier investigations on some circulatory effects of exogenous histamine before and after inhibition of histaminase (Törnqvist 1968 a and b)

In the present investigation there was a considerable increase in the concentration of histamine in both plasma and blood cell fraction after inhibition of histaminase. The lower concentrations of histamine before administration of aminoguanidine may be due either to an increased destruction of histamine *in vitro* during the time necessary for the separation of the plasma and the blood cell fraction or to an increased destruction of histamine in the circulating blood. Lindberg (1963) showed that histamine de-

before and after inhibition of histaminase. In all these cases the histaminolytic activity was decreased to a non-pregnant level after the enzyme inhibition.

In all blood samples analysed for  $^{14}\text{C}$  histamine there was a general tendency towards increased concentration of histamine after inhibition of histaminase.

Before inhibition of histaminase there was a considerable disappearance of histamine from those total blood samples which were analysed 8 minutes after sampling. No corresponding histaminolysis occurred after inhibition of histaminase.

The distribution of the recovered histamine was studied and both before and after inhibition of histaminase about 1/3-1/5 of the histamine was found in the blood cell fraction.

### Acknowledgements

This study was aided by grants from the Medical Faculty of the University of Göteborg.

I wish to thank Dr Bengt Willert M.D. for his generous help with the analyses of the histaminolytic activity in plasma.

Skilful technical assistance was given by Mrs B. Kallkopf, Miss G. Lundqvist and Mrs W. Lundström.

### REFERENCES

- Adam, H. M. *Quart. J. exp. Physiol.* 35: 281, 1950.  
Adam, H. M., Cord, W. I., Ruddell, M. I., Roberts, M. and Strong, J. A. *Brit. J. Pharmacol.* 9: 62, 1954.  
Adam, H. M., Hilderson, D. C. and Spencer, K. E. V. *Brit. J. Pharmacol.* 12: 397, 1957.  
Ahlmark, A. *Acta physiol. scandinav.* 9 suppl. 28, 1944.  
Bernius, B., Carlsson, A., Hultgren, A. and Seldinger, S. I. *Scand. J. Clin. Lab. Invest.* 6: 217, 1954.  
Brody, S., Lindberg, S. and Törnqvist, A. unpublished.  
Clark, D. H. and Tinkel, H. I. *Lancet* 2: 888, 1954.  
Dinner, H. and Pernow, B. *Scand. J. Clin. Lab. Invest.* 10: 233, 1958.  
Emanuel, N., Kahlsjö, G. and Wikström, F. *Acta physiol. scandinav.* 2: 123, 1941.  
Janzon, H. and Grossman, M. I. *Amer. J. Physiol.* 157: 94, 1949.  
Kallmark, S. *Acta endocr.* 10: 135, 1952.  
Lindberg, S. *Acta obst. et gynec. scandinav.* 42 suppl. 1: 3, 1953.

The distribution of infused isotope labelled histamine between the plasma and the blood cell fraction has been studied previously in non pregnant dogs by Lindell and Viskö (1961) and in man by Lindell and Westling (1962). In these investigations only 1/3-1/4 of the recovered histamine was found in the blood cells. The correspondance between these investigations and the present investigation is fairly good even if the increased histaminolytic activity in the pregnant women might have modified the distribution of infused histamine in the present study.

Judging by the study of the histaminolytic activity of the plasma before and after injection of aminoguanidine the method for inhibition of histaminase in the present investigation may be efficient. The histaminolytic activity was measured by the conventional method using bioassay of the remaining histamine on guinea pig ileum after incubation of plasma with non-isotopic histamine. In all the 6 pregnant women studied there was a marked reduction of histaminolytic activity after injection of aminoguanidine.

The results of the present investigation show a general tendency towards increasing concentrations of histamine in total blood plasma and blood cell fraction after inhibition of histaminase *in vivo* with otherwise identical experimental conditions. This supports the earlier observations of an increased response to infused histamine of pregnant women after inhibition of histaminase (Törnqvist 1968 a and b).

## SUMMARY

Eleven pregnant women received infusions of  $^{14}\text{C}$  histamine before and after inhibition of histaminase by means of an intramuscular injection of aminoguanidine. In all cases arterial blood was withdrawn during the infusion and after centrifugation, the concentration of isotope labelled histamine in the plasma and the blood cell fraction was estimated. In 6 of the cases arterial blood samples withdrawn simultaneously were analysed and the concentration of histamine in total blood was estimated both immediately and after 8 minutes at room temperature.

In 6 cases the histaminolytic activity of plasma was estimated

## FOREARM BLOOD FLOW DURING NORMAL PREGNANCY STUDIED BY VENOUS OCCLUSION PLETHYSMOGRAPHY AND <sup>133</sup>XENON MUSCLE CLEARANCE

BY

SVEN SPETZ AND INGE JANSSON

Marked changes in the distribution of blood flow to the skin and skeletal muscles have been shown to occur during normal pregnancy (*Abramson Flach and Flerst* 1943 *Herbert Benner and Wakim* 1954 1958 *Burt* 1950 *Spetz* 1964 *Ginsburg and Duncan* 1967)

Venous occlusion plethysmography is a convenient method for studying alterations in peripheral blood flow. This method was applied in all the above mentioned studies of skin and skeletal muscle blood flow during normal pregnancy.

Changes in total forearm blood flow measured by venous occlusion plethysmography reflect to a large extent alterations in muscular blood flow (*Grant and Pearson* 1938 *Allen Barcroft and Edholm*, 1946). Changes in skin blood flow however may also be of importance when recording variations of total forearm blood flow.

A method which made it possible to differentiate between the blood flow in different tissue components of the same organ was introduced by *Kety* in 1949 when he demonstrated that the rate of disappearance of the locally injected radioactive isotope sodium-24 was proportional to the local blood flow in the tissues. The chemically inert radioactive gases krypton-85 and xenon 133 later have proven to be even more useful in this respect (*Lassen* 1964 *Holzman Wagner Ho Rabinowitz and Zierler* 1964).

The present investigation was initially undertaken in an at

- Lindberg, S. and Törnqvist, A. *Acta obst. et gynec. scandinav* 45 131 1956
- Lindell, S. E. and Schayer, R. W. *Brit. J Pharmacol* 13 44 1958a
- Lindell, S. E. and Westling, H. Communication at the 22nd International congress of physiological sciences Leiden 1962
- Lindell, S. E. and Visker, K. *Brit. J Pharmacol* 17 131 1961
- Marcou, I. Athanasiu-Vergu, E. Chiriceanu, D. Cosma, G. Gingold, N. and Parhon, C.-C. *Presse Med.* 46 371 1938
- Rose, H. *Science* 92 454 1940
- Schayer, R. W. and Cooper, J. A. D. *J appl Physiol* 9 481 1956
- Törnqvist, A. *Acta obst. et gynec. scandinav* 47 257 1968a
- Törnqvist, A. *Acta obst. et gynec. scandinav* 47 391 1968b
- Wicksell, F. *Acta physiol. scand.* 17 359 1949

Received on April 17 1968

the repeated plethysmographic examinations could influence the following  $^{133}\text{Xe}$  clearance determination, the order was reversed and  $^{133}\text{Xe}$  clearance measured first. Furthermore the water temperature of the plethysmograph was reduced to 26 C to minimize the influence of skin blood flow on total forearm blood flow. This part of the study was used when calculating the correlation between the plethysmographic and the  $^{133}\text{Xe}$  clearance values. The water temperature used in each case is stated in Table II.

### *Plethysmography*

Venous occlusion plethysmography was applied to the right forearm in the same manner as described in detail by Spetz (1964). During measurements the proximal cuff pressure was maintained at least 20 mm Hg below the diastolic arterial pressure as determined in the left arm by the conventional sphygmomanometer and auscultation of Korotkoff's sound. In most experiments the proximal cuff pressure was 40 mm Hg. The distal cuff pressure was kept at about 60 mm Hg to prevent venous backflow from the hand and not above the arterial systolic pressure as usually recommended, because prolonged supra-arterial pressure may cause considerable pain and discomfort to the test subject. Several experiments revealed that the values for forearm blood flow were not influenced by this change in technique. At least ten consecutive measurements were performed on each test subject with intervals of about one minute. The mean value was calculated and taken as total forearm blood flow at rest, expressed in ml per 100 ml of forearm tissue per minute.

### *Xe clearance method*

$^{133}\text{Xe}$  dissolved in isotonic saline was obtained at first from the Radiochemical Centre, Amersham, England, later from AB Atomenergi, Studsvik, Nyköping, Sweden. 0.05–0.10 ml of the solution with an activity of about 30–50 microcurie was injected slowly (injection time 10–15 seconds) intramuscularly into the extensor portion of the forearm musculature. A needle no. 20 (outer diameter 0.5 mm) was used and inserted, at an oblique angle to the skin surface to a depth of about 1.5 cm. The needle

tempt to evaluate the validity of the  $^{133}\text{Xe}$  clearance method in comparison with venous occlusion plethysmography. One of us (Spetz, 1964) has demonstrated a marked increase in total forearm blood flow during the course of normal pregnancy. Thus another purpose of this study was to elucidate in which tissue component of the forearm these changes occur in the skeletal muscles in the skin or both.

### *Material*

Forty-one women who were between the 16th and 43rd weeks of pregnancy were studied. Their mean age was 25 years with a range from 16 to 44 years. The cases of early pregnancy were women about to undergo legal abortion on socio-medical grounds. Those in later pregnancy were women under observation because of some minor complication of pregnancy not affecting the circulatory system.  $^{133}\text{Xe}$  clearance in the extensor muscles of the forearm was studied in 38 subjects. In 26 of these cases forearm blood flow was studied simultaneously by venous occlusion plethysmography. In the remaining three cases only plethysmography was performed as the  $^{133}\text{Xe}$  clearance examinations had to be discarded because of technical error. Thirteen non-pregnant subjects were also studied, both methods being used in 11 of these cases. This group consisted mainly of women treated at the hospital because of salpingitis which had subsided completely at the time of examination. Their mean age was 24 years with a range from 18 to 42 years.

### *Methods*

The observations were not started until the test subjects had been resting in bed for at least 30 minutes. The room temperature was maintained at 22 C. During the earlier part of the investigation the plethysmographic examination was performed first and a water temperature of 34 C chosen as this seems to be the optimal temperature (Barcroft and Edholm 1945). About ten minutes after the arm was taken out of the plethysmograph the  $^{133}\text{Xe}$  clearance measurement was started. As we felt that



the repeated plethysmographic examinations could influence the following  $^{133}\text{Xe}$  clearance determination, the order was reversed and  $^{133}\text{Xe}$  clearance measured first. Furthermore the water temperature of the plethysmograph was reduced to 26 C to minimize the influence of skin blood flow on total forearm blood flow. This part of the study was used when calculating the correlation between the plethysmographic and the  $^{133}\text{Xe}$  clearance values. The water temperature used in each case is stated in Table II.

### *Plethysmography*

Venous occlusion plethysmography was applied to the right forearm in the same manner as described in detail by Spertz (1964). During measurements the proximal cuff pressure was maintained at least 20 mm Hg below the diastolic arterial pressure as determined in the left arm by the conventional sphygmomanometer and auscultation of Korotkoff's sound. In most experiments the proximal cuff pressure was 40 mm Hg. The distal cuff pressure was kept at about 60 mm Hg to prevent venous backflow from the hand and not above the arterial systolic pressure as usually recommended, because prolonged supra-arterial pressure may cause considerable pain and discomfort to the test subject. Several experiments revealed that the values for forearm blood flow were not influenced by this change in technique. At least ten consecutive measurements were performed on each test subject with intervals of about one minute. The mean value was calculated and taken as total forearm blood flow at rest, expressed in ml per 100 ml of forearm tissue per minute.

### *Xe clearance method*

$^{133}\text{Xe}$  dissolved in isotonic saline was obtained at first from the Radiochemical Centre, Amersham, England, later from AB Atomenergi, Studsvik, Nyköping, Sweden. 0.03–0.10 ml of the solution with an activity of about 30–50 microcurie was injected slowly (injection time 10–15 seconds) intramuscularly into the extensor portion of the forearm musculature. A needle no. 20 (outer diameter 0.5 mm) was used and inserted, at an oblique angle to the skin surface to a depth of about 1.5 cm. The needle

tempt to evaluate the validity of the  $^{133}\text{Xe}$  clearance method in comparison with venous occlusion plethysmography. One of us (Spetz 1964) has demonstrated a marked increase in total forearm blood flow during the course of normal pregnancy. Thus another purpose of this study was to elucidate in which tissue component of the forearm these changes occur in the skeletal muscles in the skin or both.

### *Material*

Forty-one women, who were between the 16th and 43rd weeks of pregnancy were studied. Their mean age was 25 years with a range from 16 to 44 years. The cases of early pregnancy were women about to undergo legal abortion on socio-medical grounds. Those in later pregnancy were women under observation because of some minor complication of pregnancy not affecting the circulatory system.  $^{133}\text{Xe}$  clearance in the extensor muscles of the forearm was studied in 38 subjects. In 26 of these cases forearm blood flow was studied simultaneously by venous occlusion plethysmography. In the remaining three cases only plethysmography was performed as the  $^{133}\text{Xe}$  clearance examinations had to be discarded because of technical error. Thirteen non pregnant subjects were also studied both methods being used in 11 of these cases. This group consisted mainly of women treated at the hospital because of salpingitis which had subsided completely at the time of examination. Their mean age was 24 years with a range from 18 to 42 years.

### *Methods*

The observations were not started until the test subjects had been resting in bed for at least 30 minutes. The room temperature was maintained at 22°C. During the earlier part of the investigation the plethysmographic examination was performed first and a water temperature of 34°C chosen as this seems to be the optimal temperature (Barcroft and Edholm 1945). About ten minutes after the arm was taken out of the plethysmograph the  $^{133}\text{Xe}$  clearance measurement was started. As we felt that

where  $\lambda$  is the muscle/blood partition coefficient of xenon. This has been calculated to be about 0.7 for skeletal muscle of the dog (Conn 1961). If this figure is taken to be valid also for human muscle tissue the original equation can be written

$$MBF = -70 \frac{dQ/dt}{Q} \text{ or}$$

$$MBF = -70 \ln 10 \frac{d \log Q}{dt}$$

where, as a logarithmic potentiometer is used,  $-d \log Q/dt$  can be expressed as the fraction of one decade which the tangent of the elimination curve decreases in one minute (D). The ultimate equation for calculating the muscle blood flow then becomes

$$MBF = 70 \cdot 2.3 D = 161 D \text{ ml per } 100 \text{ g muscle per minute}$$

(Lassen *et al.* 1964)

If the basic assumption of complete diffusion equilibrium of xenon between tissue and blood is fulfilled and the tissue area under investigation is evenly perfused, the recorded  $^{133}\text{Xe}$  clearance curve can be expected to be mono-exponential, i.e. a straight line with logarithmic recording, and D easily calculated. This was, however, very seldom the case, but in most instances the first 5–6 minutes of the recording was straight or could be approximated to a straight line (Fig. 1). Kjellmer, Lindberg, Pterovsky and Tønnesen (1967) calculated muscle blood flow from this initial slope in experiments on the isolated gastrocnemius muscle of cats and found it to correlate well with the directly measured blood flow. Zierler (1963) proposed a method of calculating blood flow from the area under the curve independent of its shape. Kjellmer *et al.* however showed that this method gave about 15 per cent lower values than the initial slope method, which in turn yielded muscle blood flow values only 62 per cent of the directly measured blood flow when intramuscular injection of the isotope was used. By a different labelling technique completely avoiding any tissue damage Tønnesen and Sejrsen recently demonstrated that the initial part of the clearance rate from a local depot of xenon is practically identical to the blood flow measured directly

was not withdrawn until 30 seconds after the injection to avoid possible backflow along the needle track. The elimination rate of the injected  $^{133}\text{Xe}$  was followed by an external scintillation detector with a NaI (TI) crystal. A cylindrical lead collimator was used with an inner diameter of 5 cm and a length of about 9 cm from the crystal to the aperture which was placed immediately above the injection site. The scintillation detector was coupled to a spectrometer set to record the peak intensity of the gamma radiation from  $^{133}\text{Xe}$  (81 KeV). A time constant of 3 seconds was chosen. The gammaspectrometer was connected to a logarithmic potentiometer writing on linear paper. The initial counting rate was usually between  $10^4$  and  $3 \cdot 10^4$  counts per minute. The clearance rate was continuously recorded during 6–12 minutes.

### Calculations

If an inert and freely diffusible substance such as xenon is introduced into a tissue it will establish equilibrium with the blood stream through the tissue very rapidly and according to the Fick principle the rate by which it is removed from the tissue will be proportional to the rate of blood flow provided the lymphatic outflow can be neglected (Kety 1960). This can be expressed with the equation

$$dQ/dt = F(C - C_v)$$

where  $Q$  is the quantity of xenon in the region at any given time ( $t$ ),  $F$  the rate of blood flow and  $C$  and  $C_v$  the concentrations of xenon in arterial and venous blood. Since the xenon is diluted by venous return from other tissues and removed almost completely from the blood via the expired air  $C$  is negligible and the equation can be reduced to

$$dQ/dt = -F C_v$$

$F$  can be substituted for  $\frac{\text{MBF} \cdot W}{100}$  where MBF is the muscle blood flow in the small area investigated per 100 g of tissue and  $W$  the tissue weight in grams.  $C_v$  can be substituted for  $\frac{Q}{W} \cdot \frac{1}{\lambda}$

where  $\lambda$  is the muscle/blood partition coefficient of xenon. This has been calculated to be about 0.7 for skeletal muscle of the dog (Conn 1961). If this figure is taken to be valid also for human muscle tissue the original equation can be written

$$MBF = -70 \frac{dQ/dt}{Q} \text{ or}$$

$$MBF = -70 \ln 10 \frac{d \log Q}{dt}$$

where as a logarithmic potentiometer is used,  $-d \log Q/dt$  can be expressed as the fraction of one decade which the tangent of the elimination curve decreases in one minute (D). The ultimate equation for calculating the muscle blood flow then becomes

$$MBF = 70 \cdot 2.3 D = 161 D \text{ ml per 100 g muscle per minute (Lassen et al. 1964)}$$

If the basic assumption of complete diffusion equilibrium of xenon between tissue and blood is fulfilled and the tissue area under investigation is evenly perfused, the recorded  $^{133}\text{Xe}$  clearance curve can be expected to be mono-exponential i.e. a straight line with logarithmic recording, and D easily calculated. This was, however, very seldom the case but in most instances the first 5-6 minutes of the recording was straight or could be approximated to a straight line (Fig. 1). Kjellmer, Lindberg, Pirovsky and Tonnesen (1967) calculated muscle blood flow from this initial slope in experiments on the isolated gastrocnemius muscle of cats and found it to correlate well with the directly measured blood flow. Zierler (1965) proposed a method of calculating blood flow from the area under the curve independent of its shape. Kjellmer et al. however showed that this method gave about 15 per cent lower values than the initial slope method, which in turn yielded muscle blood flow values only 62 per cent of the directly measured blood flow when intramuscular injection of the isotope was used. By a different labelling technique, completely avoiding any tissue damage, Tonnesen and Sejsen recently demonstrated that the initial part of the clearance rate from a local depot of xenon is practically identical to the blood flow measured directly

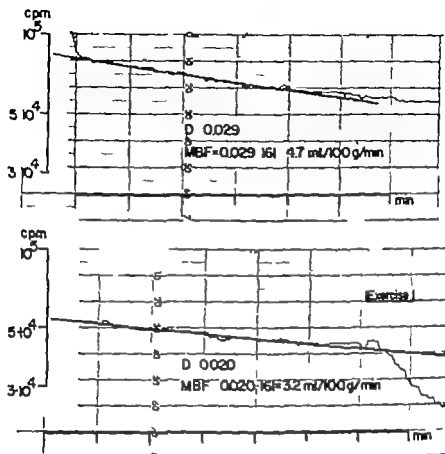


Fig. 1 Original charts of two recordings of  $^{133}\text{Xe}$  clearance from forearm muscle with lines drawn in to illustrate the straight part of the curve. At the end of the lower recording the test subject produced hyperaemia by one minute's muscular exercise.

(Tonnesen 1968). Thus MBF was calculated from the equation given above. When drawing the line of slope care was taken to include as much as possible of the initial part of the curve. Curves with a very marked multi-exponential course were discarded because it was impossible to make a straight line fit the curve. No attempt was made to divide the curves into two or more components as described by e.g. Haggendal Nilsson and Norbäck (1965) for brain tissue or Lundgren (1967) for small intestine as the meaning of this in the case of skeletal muscle is obscure.

Table 1. Forearm Blood Flow Measured by Plethysmography (Water Temperature 26°C) and  $^{133}\text{Xe}$  Clearance in Forearm Muscle in Non-Pregnant Subjects

Subj. No.	Blood Flow Plethysm. ml/100 ml/min $\pm$ SE	$^{133}\text{Xe}$ Clearance Slope/min (D)	Flow ml/100 g/min
57	3.9 $\pm$ 0.22	0.028	4.5
58	1.8 $\pm$ 0.08	0.018	2.9
59	2.2 $\pm$ 0.16	0.014	2.3
60	3.9 $\pm$ 0.27	0.023	3.8
62	3.7 $\pm$ 0.08	0.029	4.7
66	1.4 $\pm$ 0.04	0.018	2.6
69	1.2 $\pm$ 0.06	0.017	2.7
70	3.3 $\pm$ 0.18	0.019	3.4
78	1.4 $\pm$ 0.02	0.018	2.8
79	2.8 $\pm$ 0.10	0.023	3.6
83	2.0 $\pm$ 0.06	0.016	2.6
90		0.020	3.2
92		0.015	2.4
Mean $\pm$ SE	2.5 $\pm$ 0.32		3.2 $\pm$ 0.22

### Statistical methods

The mean value standard error of the mean (SE) correlation coefficient and regression line were calculated by conventional methods (Brownlee 1965). In the text and tables the mean value is given  $\pm$  SE. The significance of differences was evaluated by the *t* test. When the variances were unequal the Welch procedure was applied (Brownlee 1965). When not otherwise stated the tests were performed at the 5 per cent level of significance.

### Results

The results in the non-pregnant subjects are given in Table 1. The mean forearm blood flow recorded with the plethysmograph was  $2.5 \pm 0.32$  ml/100 ml/min and the mean  $^{133}\text{Xe}$  clearance in forearm muscle  $3.2 \pm 0.22$  ml/100 g/min. Comparison by a

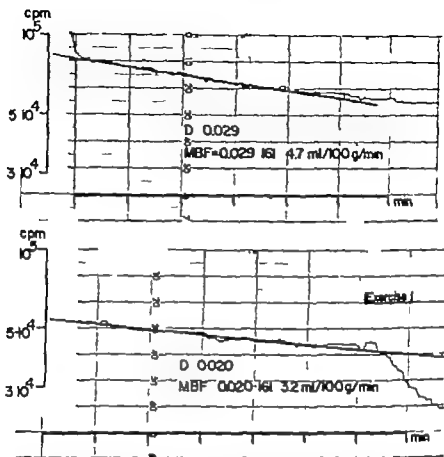


Fig 1 Original charts of two recordings of  $^{146}\text{Xe}$  clearance from forearm muscle with lines drawn in to illustrate the straight part of the curve. At the end of the lower recording the test subject produced hyperaemia by one minute's muscular exercise.

(Tonnesen 1968). Thus MBF was calculated from the equation given above. When drawing the line of slope care was taken to include as much as possible of the initial part of the curve. Curves with a very marked multi-exponential course were discarded because it was impossible to make a straight line fit the curve. No attempt was made to divide the curves into two or more components as described by e.g. Häggendal Nilsson and Norbäck (1965) for brain tissue or Lundgren (1967) for small intestine as the meaning of this in the case of skeletal muscle is obscure.



Table III. Total Forearm Blood Flow and  $^{133}\text{Xe}$  Clearance in Forearm Muscle in Non-Pregnant Subjects and at Different Periods of Pregnancy Mean Values  $\pm$  Standard Error of Mean

Method	Non-Preg- nant Subjects	16-24 Weeks of Pregnancy	25-36 Weeks of Pregnancy	37-43 Weeks of Pregnancy
Plethysm.	$2.9 \pm 0.32$	$3.5 \pm 0.66$	$5.6 \pm 0.42$	$7.7 \pm 0.76$
ml/100 ml/min	n=11	n=9	n=7	n=13
$^{133}\text{Xe}$ clearance	$3.2 \pm 0.21$	$4.3 \pm 0.54$	$3.1 \pm 0.64$	$3.3 \pm 0.22$
ml/100 g/min	n=13	n=13	n=5	n=20

t test on differences between measurements in the same case shows that the difference is statistically significant.

The results in the pregnant subjects are collected in Table II. The mean total forearm blood flow of all pregnant women regardless of gestation period was  $5.9 \pm 0.52$  ml/100 ml/min. This value is significantly higher than that found in the non pregnant subjects ( $P < 0.001$ ). The mean  $^{133}\text{Xe}$  clearance rate in forearm muscle in the pregnant women was  $3.6 \pm 0.24$  ml/100 g/min. This is not significantly different from the mean  $^{133}\text{Xe}$  clearance rate in the non-pregnant group.

In Table III the mean values for resting forearm blood flow and for  $^{133}\text{Xe}$  muscle clearance at different periods of pregnancy are given. The mean forearm blood flow recorded by plethysmograph between the 16th and 24th weeks of pregnancy was not significantly different from that of non-pregnant subjects. The mean values for total forearm blood flow between the 25th and 36th, and the 37th and 43rd weeks of pregnancy were both significantly higher than the mean value of the non-pregnant group ( $P < 0.001$ ). There was, however, no significant difference between the mean values of muscle blood flow determined by  $^{133}\text{Xe}$  clearance at different periods of gestation when compared with the non-pregnant group.

In Fig. 2 the mean values are plotted against the duration of pregnancy. It is evident from the figure that the forearm blood flow recorded with the plethysmograph and the  $^{133}\text{Xe}$  clearance in forearm muscle were about equal until about the 24th week

Table II. Forearm Blood Flow Measured by Plethysmography and  $^{133}\text{Xe}$  Clearance in Forearm Muscle in Healthy Pregnant Women

Subj No	Week of Pregnancy	Water Temp Pleth C	Blood Flow Plethysm. ml/100 ml/min $\pm$ SE	$^{133}\text{Xe}$ Clearance	
				Slope/min (D)	Flow ml/100 g/min
2	16			0.026	4.3
25	16	34	$2.5 \pm 0.05$	0.040	6.4
6	17			0.034	5.4
5	18			0.016	2.6
65	18	26	$7.6 \pm 0.24$	0.051	8.3
89	18			0.013	2.1
84	19	26	$4.1 \pm 0.17$	0.034	5.5
32	20	34	$3.1 \pm 0.12$	0.019	3.1
73	20	26	$2.6 \pm 0.20$	0.019	3.1
63	21	26	$4.0 \pm 0.12$	0.028	4.4
72	21	26	$1.3 \pm 0.05$	0.009	1.5
61	22	26	$1.4 \pm 0.03$	0.019	3.1
76	22	26	$5.1 \pm 0.29$	0.036	5.7
68	25	26	$6.2 \pm 0.28$		
34	26	34	$5.4 \pm 0.17$	0.020	4.8
80	30	26	$5.2 \pm 0.37$	0.012	1.9
85	30	26	$3.6 \pm 0.19$	0.011	1.8
53	31	34	$5.0 \pm 0.28$		
46	32	34	$6.8 \pm 0.18$	0.017	2.7
77	35	26	$6.7 \pm 0.59$	0.029	4.7
45	37	34	$9.9 \pm 0.31$	0.029	4.7
49	37	4	$6.1 \pm 0.16$	0.010	1.6
87	37			0.020	3.2
21	38			0.015	2.4
71	38	26	$2.4 \pm 0.16$	0.017	2.7
81	38	26	$5.4 \pm 0.18$	0.020	3.2
8	39			0.023	3.7
54	39	34	$10.6 \pm 0.40$	0.020	3.2
67	39	26	$9.3 \pm 0.21$	0.027	4.3
74	39	26	$9.4 \pm 0.27$	0.022	3.5
82	39	26	$10.7 \pm 0.40$	0.017	2.7
86	39	26	$4.4 \pm 0.21$	0.025	4.0
88	39			0.014	2.2
31	40			0.020	3.2
43	40	34	$10.3 \pm 0.57$	0.030	4.8
56	40	26	$5.0 \pm 0.21$	0.034	5.5
75	40	26	$8.5 \pm 0.53$	0.018	2.9
91	40			0.013	2.1
52	41	34	$6.0 \pm 0.22$		
3	43			0.025	4.0
19	43			0.018	2.9
Mean $\pm$ SE			$5.9 \pm 0.52$		$3.6 \pm 0.24$

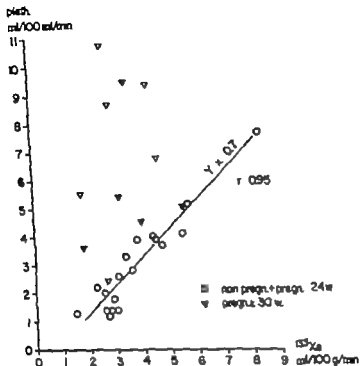


Fig 3 Relation between total forearm blood flow determined by plethysmography and  $^{133}\text{Xe}$  clearance in forearm muscle in pregnant and non-pregnant subjects

and pregnant cases up to 22 weeks of gestation, the other of cases 30 weeks pregnant or more (there were no cases between 23 and 29 weeks in this series) an interesting pattern appears. For the first group there is a linear correlation between the plethysmographic forearm blood flow and the  $^{133}\text{Xe}$  muscle clearance the correlation coefficient ( $r$ ) being 0.95 ( $P < 0.001$ ). The calculated regression line is  $Y = 1.07X - 0.7$  meaning that the muscle blood flow calculated from the  $\text{Xe}$  clearance rate was on a average 0.7 ml/min. higher than the total forearm blood flow determined by plethysmography but that an increase of one unit in blood flow with one method meant an increase of one

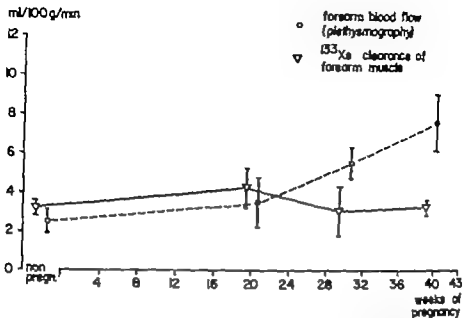


Fig. 2. Forearm blood flow determined by plethysmography and  $^{133}\text{Xe}$  clearance in forearm muscle in non-pregnant subjects and at different stages of pregnancy. The mean values are given  $\pm 2$  SE.

of pregnancy. From then on the total forearm blood flow showed a progressive rise while the  $^{133}\text{Xe}$  clearance remained fairly constant throughout the pregnancy.

### Correlation study

In this part of the study were included all pregnant and non-pregnant cases where the water temperature of the plethysmograph was maintained at 26°C and the plethysmographic as well as the Xe clearance recordings were considered accurate. There were 29 such pairs of observations. In Fig. 3 the relationship between blood flow values determined by the two methods is illustrated. There seemed at first to be a fairly uniform distribution of the plotted values in the coordinate system and no definite correlation could be established. If however the series is divided into two groups, one composed of all non-pregnant cases

ml/min. This difference may perhaps be explained by technical differences in performing the forearm blood flow measurements.

In non-pregnant subjects total forearm blood flow is mainly muscle blood flow as the muscles occupy about 60 per cent of the forearm tissue volume and the skin and subcutaneous tissue only about 10 per cent (Abramson and Ferris 1940 Cooper Edholm and Mortram 1955). The rest of the forearm volume is bones and tendons, the blood flow in which is low and constant and can be neglected. Changes in hand blood flow however reflect to a large extent changes in skin blood flow as 30 per cent of the hand volume is skin and only 16 per cent muscle (Abramson and Ferris 1940). There is general agreement (Burr 1930 Spetz 1965 Ginsburg and Duncan 1967) that there is an increase in skin blood flow in late pregnancy of about 15 ml/100 ml/min as judged from hand plethysmography.

Thus skin blood flow must make a contribution to the rise in total forearm blood flow in late pregnancy but it seemed to us improbable that it could be responsible for the whole increase. Kety's (1949) tissue clearance method to determine local blood flow seemed suitable to evaluate the part played by the muscle mass in this increase. Kety originally used  $^{222}\text{Rn}$  but the radioactive inert gas  $^{133}\text{Xe}$  later has been shown to give more reliable information (Lassen 1964) as it is freely diffusible through the cell membrane and can be assumed to establish equilibrium between tissue and blood very rapidly. Lassen Lindbjerg and Munk (1964) and Lassen Lindbjerg and Dahn (1965) applied the  $^{133}\text{Xe}$  clearance method to the skeletal muscles of the leg in cases of occlusive vascular disease and found the method to permit a clearcut differentiation between normal and diseased legs. Recently Siggaard Andersen and Bonde Petersen (1967) and Tonnesen (1968) have demonstrated a good correlation between  $^{133}\text{Xe}$  muscle clearance and plethysmography of the calf under conditions of submaximal vasodilatation.

When the  $^{133}\text{Xe}$  clearance method was applied to the forearm muscles during pregnancy no change in the calculated muscle blood flow could be demonstrated, despite the rise in total forearm blood flow during the second half of pregnancy. However it was possible to demonstrate a strong linear correlation ( $r =$

unit also with the other. After the 30th week of pregnancy the total forearm blood flow did not show any correlation with  $^{133}\text{Xe}$  muscle clearance.

### Discussion

Changes in peripheral circulation during pregnancy as reflected by total forearm blood flow have been studied by a number of investigators. *Abramson et al* (1943) and *Ginsburg and Duncan* (1967) were not able to demonstrate any change in resting forearm blood flow during pregnancy. The latter authors however found a progressive increase in hand blood flow towards the end of pregnancy. *Burt* (1950) made this latter observation but she also noted an increase in forearm blood flow as did *Herbert et al.* (1954, 1958).

One of us (*Spetz* 1964) has studied forearm blood flow at different stages of normal pregnancy under strictly standardized conditions by means of venous occlusion plethysmography. A progressive increase in forearm blood flow was shown to occur following an exponential function from midpregnancy until term. The mean forearm blood flow in early pregnancy was 2.8 ml/100 ml/min as compared with 12.3 ml at term, meaning a four to fivefold increase.

The present study has confirmed the finding by *Spetz* (1964). The rise in forearm blood flow during the second half of pregnancy however was not of the same magnitude. This may be explained by differences in water temperature of the plethysmograph. *Spetz* used a temperature of 36°C while in the present study lower temperature levels 34°C and 26°C were chosen. At 34°C the resting mean forearm blood flow at term was  $9.0 \pm 0.9$  ml/100 ml/min ( $n=5$ ) and at 26°C  $6.9 \pm 1.0$  ml/100 ml/min ( $n=8$ ). The difference suggests that the lower water temperature by reducing skin blood flow brought about a decrease in total forearm blood flow. Nevertheless there was a more than threefold increase in peripheral blood flow at the end of pregnancy. This result is in contrast with the findings of *Ginsburg and Duncan* (1967) who reported a remarkably low forearm blood flow throughout the pregnancy 1.9–2.7 ml/100

must be assumed to take place through open capillaries not involved in blood-tissue exchange. As xenon is freely diffusible it should be independent of a pressure gradient across the capillary wall. Thus, if a non-nutritional capillary blood flow exists,  $^{133}\text{Xe}$  clearance should measure this too. There is however the possibility of unequal perfusion of different parts of the same muscle, in which case  $^{133}\text{Xe}$  clearance would not reflect the conditions in the entire muscle.

A comment of the second alternative requires some mathematical considerations. As skin constitutes only 30 per cent of the hand volume an increase of 15 ml/100 ml/min in hand blood flow must mean a rise in skin blood flow of about 50 ml per min and 100 ml skin, neglecting the blood flows of the other tissues of the hand. If this figure is applied to the skin of the forearm, which occupies about 10 per cent of the forearm volume, it can account for a rise in total forearm blood flow of about 5 ml/100 ml/min. This is very close to the actually observed increase in the present study.

Thus, the results of the present study are in favour of an increased skin blood flow being the cause of the observed rise in forearm blood flow during late pregnancy. This conclusion, however, is based on the assumption that the change in forearm skin blood flow is equal to that of the hand. This may not be true. In any case a general increase in skin blood flow of that magnitude would mean that the skin of the body would take an unreasonably great share of the cardiac output at the end of pregnancy.

## SUMMARY

1 Resting forearm blood flow determined by venous occlusion plethysmography and  $^{133}\text{Xe}$  clearance in the extensor musculature of the forearm was studied in forty-one pregnant and thirteen non-pregnant healthy subjects.

2 The mean forearm blood flow recorded with the plethysmograph in the non-pregnant group was 2.5 ml, in midpregnancy 3.5 ml and in late pregnancy 7.7 ml/100 ml/min. The corresponding mean  $^{133}\text{Xe}$  clearance values of forearm muscle was 3.2, 4.3

0.95) between forearm blood flow recorded by the plethysmograph and  $^{133}\text{Xe}$  clearance of forearm muscles in non pregnant and early pregnant subjects. This indicates that  $^{133}\text{Xe}$  clearance reflects individual variations in forearm muscle blood flow under these circumstances.

To be able to discuss these contradictory results it is necessary to review the current conception of skeletal muscle blood flow (cf Folkow 1967). From a functional point of view each vascular circuit in the body consists of various series-coupled sections: the local and central nervous regulation of which may vary considerably. 1) "Windkessel" vessels (large and middle sized arteries), 2) resistance vessels with a precapillary and a postcapillary section, 3) exchange vessels, i.e. true capillaries with their precapillary sphincter vessels and ultimately 4) capacitance vessels (venules and veins). In addition shunt vessels have been demonstrated in some tissues e.g. in skin. The tone of the resistance vessels determines the peripheral resistance to blood flow and hence the magnitude of regional blood flow. This is measured by venous occlusion plethysmography. The tone and relation between pre- and postcapillary resistance vessels determine to a great extent mean hydrostatic capillary pressure and the rate of transcapillary filtration. Changes in tone of the precapillary sphincter vessels regulate the number of capillaries open for blood flow and capillary exchange. As the locally injected tracer has to be eliminated through perfused capillaries  $^{133}\text{Xe}$  clearance must be assumed to measure the rate of blood flow in these capillaries. This capillary blood flow is mainly regulated by the local metabolic demands of the tissues.

Thus in the non pregnant and early pregnant state this capillary blood flow is equal to the total regional blood flow. The results in late pregnancy suggests that the total regional blood flow then greatly exceeds the capillary blood flow of the muscles. This may have two possible explanations. The first is that blood may pass through vessels not involved in muscle nutrition. The second is that the rise in skin blood flow is greater than supposed.

As there are no true arterio-venous anastomoses in muscle tissue (Walder 1968) any shunting of blood that may occur



must be assumed to take place through open capillaries not involved in blood-tissue exchange. As xenon is freely diffusible it should be independent of a pressure gradient across the capillary wall. Thus if a non-nutritional capillary blood flow exists,  $^{133}\text{Xe}$  clearance should measure this too. There is, however the possibility of unequal perfusion of different parts of the same muscle, in which case  $^{133}\text{Xe}$  clearance would not reflect the conditions in the entire muscle.

A comment of the second alternative requires some mathematical considerations. As skin constitutes only 30 per cent of the hand volume an increase of 15 ml/100 ml/min in hand blood flow must mean a rise in skin blood flow of about 50 ml per min and 100 ml skin, neglecting the blood flows of the other tissues of the hand. If this figure is applied to the skin of the forearm, which occupies about 10 per cent of the forearm volume, it can account for a rise in total forearm blood flow of about 5 ml/100 ml/min. This is very close to the actually observed increase in the present study.

Thus, the results of the present study are in favour of an increased skin blood flow being the cause of the observed rise in forearm blood flow during late pregnancy. This conclusion, however, is based on the assumption that the change in forearm skin blood flow is equal to that of the hand. This may not be true. In any case a general increase in skin blood flow of that magnitude would mean that the skin of the body would take an unreasonably great share of the cardiac output at the end of pregnancy.

## SUMMARY

1 Resting forearm blood flow determined by venous occlusion plethysmography and  $^{133}\text{Xe}$  clearance in the extensor musculature of the forearm was studied in forty-one pregnant and thirteen non-pregnant healthy subjects.

2 The mean forearm blood flow recorded with the plethysmograph in the non pregnant group was 2.5 ml, in midpregnancy 3.5 ml and in late pregnancy 7.7 ml/100 ml/min. The corresponding mean  $^{133}\text{Xe}$  clearance values of forearm muscle was 3.2, 4.3

and 3.3 ml/100 g/min. The total regional blood flow of the forearm as determined by plethysmography showed a progressive rise from midpregnancy until term, while  $^{125}\text{I}$  clearance in forearm muscle was unchanged throughout the pregnancy and equal to that of non pregnant subjects

3 There was a strong positive correlation ( $r=0.95$ ) between forearm blood flow determined by plethysmography and  $^{125}\text{I}$  muscle clearance when the groups of non pregnant subjects and pregnant women up to 22 weeks of gestation were taken together. In late pregnancy no relationship between blood flow values determined by the two methods could be demonstrated. The reason for this is discussed.

### *Acknowledgements*

This study was aided by grants from the Medical Faculty of the University of Goteborg, the Swedish Medical Research Council and the Medical Society of Goteborg. The statistical analysis was performed by Mrs Gull Britt Palm fil kand

### REFERENCES

- Abramson D I and Ferris E B *Amer Heart J* 19 541 1940  
 Abramson D I Flachs A and Ferris S M *Amer J Obstet Gynec* 45 666, 1943  
 Allen W J Barcroft H and Edholm O G *J Physiol* 105 255 1946  
 Barcroft H and Edholm O G *J Physiol* 104 366 1945  
 Brownlee K A *Statistical Theory and Methodology in Science and Engineering*, New York 1955  
 Burt C C *Edinburgh Med. J Trans Edinb Obstet Soc* 57 19 1950  
 Con H L *J Appl Physiol* 16 1065 1961  
 Cooper K E Edholm O G and Mottram R R *J Physiol* 129 255 1955  
 Ginsburg J and Duncan S *Cardiovasc Res* 1 132, 1957  
 Grant R T and Pearson R S III *Clin Sci* 3 119 19 8  
 Folkow B *Triangle* 8 0 19 7  
 Herbert C M Banner E A and Wilkin K G *Amer J Obstet Gynec* 68 1553 1954  
 Herbert C M Banner E A. and Wilkin K G *Amer J Obstet Gynec* 76 742 1958  
 Holzman G III Wagner H V J I M Rabinowitz D and Zierler K. L. *Circulation* 33 27 1964

- Hägerdal E Nilsson N J and Norbäck B *Acta Physiol Scand.* 66 suppl. 258 5 1965
- Kety S S *Arter Heart J* 38 321 1949
- Kety S S. *Metb. Med. Res.* 8 223, 1950
- Kjellmer I Lindberg, I Pterovsky, I and Tønnesen H *Acta Physiol. Scand.* 69 69 1957
- Lessem N A. *J Clin. Invest.* 43 1805 1964
- Lessem N A. Lindberg, I and Dahn, L. *Circulat. Res.* 16 207 1965
- Lessem N A Lindberg, I and Munch, O. *Lancet* 1 686 1964
- Lundgren O *Acta Physiol. Scand.*, suppl. 303 1967
- Sjögård-Andersson J and Bonde Petersen F *Scand. J clin. Lab. Invest.* 19 106 1957
- Spetz S. *Acta Obstet. Gynec. Scand.* 43 309 1964
- Spetz S *Acta Obstet. Gynec. Scand.* 44 suppl. 1 1965
- Tønnesen H *Scand. J clin. Lab. Invest.* 21 65 1968
- Walden D W in *Circulation in Skeletal Muscle*, Ed. Hudlická Pergamon Press Oxford, 1968 p 101
- Zurier K L. *Circulat. Res.* 16 309 1955

Received on Aug. 8 1968

## <sup>131</sup>XENON CLEARANCE IN THE MYOMETRIUM OF PREGNANT AND NON PREGNANT WOMEN

BY

INGE JANSSON

The complicated vascular anatomy of the uterus makes direct measurements of uterine blood flow during human pregnancy extremely difficult. The electromagnetic flowmeter used by *Assali Rauramo and Peltonen* (1960) measures the blood flow only in one uterine artery and its application implies considerable risks and uncertainties. The total uterine blood flow per unit weight of tissue has been calculated from the Fick principle using nitrous oxide (*Assali Douglass Baird Nicholson and Suyemoto* 1953 *Metcalf Romney Ramsey Reid and Burwell* 1955) or 4-aminoantipyrine (*Huckabee* 1962) as test substances. This technique however is based on repeated sampling of blood from a representative uterine vein, involving hazardous catheterization procedures or venous cannulation at caesarean section.

Kety's (1949) method of measuring regional blood flow from the disappearance rate of a locally injected radioactive tracer ( $^{24}\text{Na}$ ) made a less traumatic approach possible. This method has been applied to the pregnant uterus by several investigators (*Broune and Veall* 1953 *Morris Osborn and Payling Wright* 1955 *Johnson and Clayton* 1957 *Moore and Myerscough* 1957 *Dixon Browne and Davey* 1963) all demonstrating a reduced  $^{24}\text{Na}$  clearance in the myometrium in preeclampsia in comparison with normal pregnancy. Their results have been criticized for a number of reasons (*Huckabee* 1962 *Hyttén and Leitch* 1964 *Assali and Morris* 1964). One objection is that the tracer is de-

posited in a very small area assuming homogeneity of the tissue, and another one that the clearance rate is proportional not only to the blood flow but also to the diffusion rate of the tracer across the capillary membrane. While some of the criticism is applicable also to  $^{133}\text{Xe}$ , a radioactive gas which in recent years has replaced  $^{24}\text{Na}$  in measurements of local tissue blood flow (Lassen Lindbjerg and Munck 1964) this tracer has one definite advantage over  $^{24}\text{Na}$ . It is chemically inert and freely diffusible through the cell membranes allowing calculation of tissue clearance in absolute figures of local blood flow. Furthermore in spite of local injection of a small depot of the tracer the  $^{133}\text{Xe}$  clearance rate has been shown to reflect regional blood flow in skeletal muscle relatively well (Kjellmer et al 1967 Tonnesen 1968 Spetz and Jansson 1969).

$^{133}\text{Xe}$  has been used to measure myometrial blood flow in late pregnancy by Munck Lysgaard Pontonnier Lefèvre and Lassen (1964) Lysgaard and Lefèvre (1965) and by Guilhem Pontonnier and Pontonnier (1965). As had earlier been the experience with  $^{24}\text{Na}$ , they found a wide scatter of the recorded clearance values. In a methodological study on cases of legal abortion Falk Forskman and Lindell (1967) could demonstrate that this scatter was largely dependent of the distance of the injection site from the placenta.

The present study was undertaken in order to reveal possible variations of  $^{133}\text{Xe}$  clearance in the myometrium at different periods of pregnancy in each case determining the site of the placenta. As a comparison myometrial  $^{133}\text{Xe}$  clearance in the non-pregnant state and in the puerperium was studied.

### Material

Forty-six measurements of myometrial  $^{133}\text{Xe}$  clearance were performed in 40 women between the 10th and 43rd weeks of pregnancy. Their ages varied from 19 to 43 years. Twenty measurements in the first half of pregnancy were carried out in 17 women submitted to the clinic for legal abortion on psychiatric or gynaecological grounds. In 13 of these cases sterilization was also performed and the placental site could be determined at

## **<sup>131</sup>XENON CLEARANCE IN THE MYOMETRIUM OF PREGNANT AND NON PREGNANT WOMEN**

BY

INGE JANSSON

The complicated vascular anatomy of the uterus makes direct measurements of uterine blood flow during human pregnancy extremely difficult. The electromagnetic flowmeter used by *Assali Rauramo and Peltonen* (1960) measures the blood flow only in one uterine artery and its application implies considerable risks and uncertainties. The total uterine blood flow per unit weight of tissue has been calculated from the Fick principle using nitrous oxide (*Assali Douglass Baird Nicholson and Suyemoto* 1953 *Metcalf Romney Ramsey Reid and Burwell* 1955) or 4-aminoantipyrine (*Huckabee* 1962) as test substances. This technique however is based on repeated sampling of blood from a representative uterine vein involving hazardous catheterization procedures or venous cannulation at caesarean section.

*Kety's* (1949) method of measuring regional blood flow from the disappearance rate of a locally injected radioactive tracer ( $^{24}\text{Na}$ ) made a less traumatic approach possible. This method has been applied to the pregnant uterus by several investigators (*Browne and Veall* 1953 *Morris Osborn and Payling-Wright* 1955 *Johnson and Clayton* 1957 *Moore and Myerscough* 1957 *Dixon Browne and Davey* 1963) all demonstrating a reduced  $^{24}\text{Na}$  clearance in the myometrium in preeclampsia in comparison with normal pregnancy. Their results have been criticized for a number of reasons (*Huckabee* 1962 *Hytten and Lelich* 1964 *Assali and Morris* 1964). One objection is that the tracer is de

cases where a clearcut differentiation of injection site could be obtained between placental and non-placental myometrium (myometrium proper) were included in the study

This method of transperitoneal injection through the exposed peritoneum was also used in three puerperal cases, where sterilization was performed.

In the non-pregnant cases the uterus had to be exposed during the injection but was well covered by adjacent structures in the pelvis during the measurement.

2. *Cases with placental localisation by arteriography* The test subject was resting in bed for at least 30 minutes before the measurement was started. As the placental site was known, a suitable place for the injection of xenon could be chosen, either placental myometrium or myometrium proper. The injection was performed transabdominally with the same thin needle as described above and without anaesthesia, as the penetration of the needle caused very little or no discomfort. When the uterine wall was reached the needle point was inserted about 5-10 mm further into the myometrium. The injection technique was the same as described above and the elimination rate of the injected <sup>133</sup>Xe recorded in the same way. An identical procedure was used in those cases where the placental site was not known and in 7 of the puerperal cases.

The *admission dose* for each measurement is extremely low. Lassen (1965) calculated that 100 Ci <sup>133</sup>Xe exposed the foetus and ovaries to a dose of 0.4 millirad at a distance of 10 cm.

*Calculations* Assuming a tissue/blood partition coefficient of xenon for myometrium of 0.7 which is stated to be an approximately correct figure for most tissues except fat (Andersen and Laefoged 1967) the local myometrial blood flow (MBF) can be calculated from the equation given by Lassen et al (1964)

$$MBF = 161 \cdot D \text{ ml per } 100 \text{ g of tissue per minute.}$$

1) the fraction of one decade by which the logarithmic elimination curve decreases in one minute. With an even perfusion of the tissue the elimination curve should form a straight line. This was almost always the case in measurements on non-pregnant and puerperal uteri but in experiments on pregnant uteri the curve often showed a multi-exponential course. Most often, how

**hysterotomy** Twenty six measurements were undertaken in 23 women who were 25–43 weeks pregnant. In 12 of these cases the placenta was located by pelvic arteriography in 5 cases at elective caesarean section. The indication for placentography was slight to moderate ante partum bleeding with suspicion of placenta praevia. Complete placenta praevia was diagnosed in one case and a minor degree in three others. The bleeding had subsided completely in all cases at the time of the  $^{133}\text{Xe}$  clearance examination.

Eleven non pregnant women, aged 17–40 years, were investigated during abdominal operations for ovarian cysts or sterilization. In all these cases the uterus looked normal. Ten women, aged 22–41 years were studied in the immediate post partum period.

### Methods

1 *Laparotomy cases* (legal abortions with sterilization, caesarean sections non pregnant cases) Intubation anaesthesia with controlled breathing through a non-rebreathing system was used in order to make recirculation of xenon negligible. A low midline incision was made down to the peritoneum, which was not incised. About 50  $\mu\text{Ci}$   $^{133}\text{Xe}$  dissolved in 0.10–0.15 ml isotonic saline was injected through the intact peritoneum into the adjacent uterine wall. The needle 5 cm long with an outer diameter of 0.5 mm, was introduced at an oblique angle to the uterine wall to a depth of about one cm. No aspiration was made and care was taken to inject the solution slowly (injection time at least 10 seconds) and not to withdraw the needle until 15–30 seconds after the injection was ended. The gamma-radiation from the injected isotope was recorded by the same set of scintillation detector, gammaspectrometer and logarithmic potentiometer writing on linear paper described in an earlier paper (Spet- and Jansson 1969). The aperture of the lead collimator covered with sterile draping, was placed immediately above the injection site. When the measurement was completed the abdominal cavity was entered and the relation between the injection site and the placenta was determined at the subsequent hysterotomy. Only those



Table I. Reproducibility of <sup>133</sup>Xe Clearance Method

Case		<sup>133</sup> Xe Clearance ml/100 g/min		
		1st Determ.	2nd Determ.	Difference
N.F.	non-pregnant	25.8	28.6	-2.8
K.A.	non-pregnant	17.3	15.9	+1.4
L.O.	non-pregnant	15.7	14.2	+1.5
E.A.	non-pregnant	9.0	8.2	+0.8
B.N.	non-pregnant	40.5	39.7	+0.8
A.O.	non-pregnant	28.8	20.0	+8.8
M.N.	pregnant	20.2	24.6	-4.4
R.J.	puerperal	43.0	38.6	+4.4

involved in such a procedure in this type of tissue. For a more detailed discussion of the calculation of blood flow from the slope of the elimination curve cf. Sperz and Jansson (1969).

**Statistical methods.** Conventional statistical methods were used for calculation of mean value and standard error of the mean (SE). In the text and tables the mean values are given  $\pm$  SE. The significance of differences between groups was tested by one way analysis of variance and Scheffé's method for multiple comparisons. Bartlett's test was used to test the equality of variances. For details see Brownlee (1965). When not otherwise stated the tests were performed at the 5 per cent level of significance.

The reproducibility of the <sup>133</sup>Xe clearance method was evaluated by a test on squared differences between paired observations (Table I). The difference between two observations in the same case is not statistically different from zero.

## Results

### Non pregnant women

The mean of 18 measurements of myometrial <sup>133</sup>Xe clearance in 11 women was  $23.0 \pm 2.98$  ml/100 g/min (Table II). The range was as great as 8.2-40.5 ml/100 g/min. There was no difference in clearance rate between different parts of the uterine body as can be seen from Table II and Fig. 2. The reason for the great variation could not be demonstrated in this small series. It is, however, interesting to note that the mean myometrial <sup>133</sup>Xe

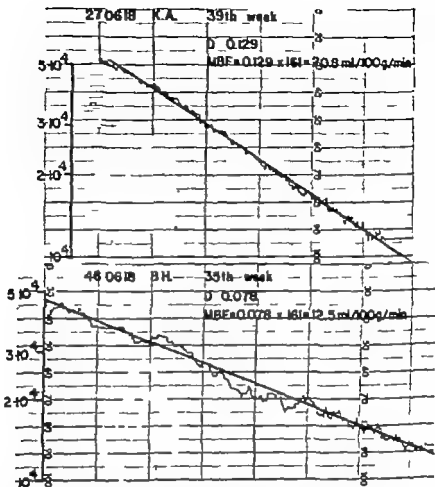


Fig. 1 Myometrial  $^{133}\text{Xe}$  clearance in two pregnant women. In both cases the placenta was located by pelvic arteriography. In the upper recording the placenta was situated in the anterior wall where the xenon injection was made. In the lower recording the placenta was situated in the posterior wall and the xenon injection made in the anterior wall i.e. in myometrium proper. The ordinate denotes the externally recorded gamma-radiation expressed in counts per minute (cpm). The distance between each heavy vertical line is one minute.

ever the initial part of the curve was satisfactorily straight until 70–80 per cent of the injected  $^{133}\text{Xe}$  was eliminated. The myometrial blood flow was then calculated from this initial slope (Fig. 1). If it was impossible to make a straight line fit the curve the experiment was discarded. No attempt was made to divide the curves into different components because of the uncertainty



26 1119 NLF

FUNDUS

CORPUS

ISTHMUS

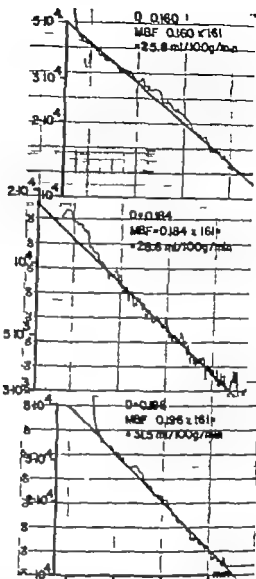


Fig. 2. Three recordings of myometrial  $^{133}\text{Xe}$  clearance in the same non-pregnant uterus. The figure illustrates the reproducibility of the clearance method and the fact that there is no essential difference in clearance rate between different parts of the non-pregnant uterus. The ordinates denote gamma-radiation in cpm.

clearance in 7 parous women was 27.6 ml/100 g/min as compared with 15.1 ml in 4 non parous women. The lowest recorded value 8.2 ml/100 g/min, was found in a 17 years old gravida with a small juvenile uterus.

Table II.  $^{133}\text{Xe}$  Clearance in Myometrium in Non-Pregnant Women

Patient Name	Age, Years	No. of Pregnancies	Day of Menstr. Cycle	$^{133}\text{Xe}$ Clearance			Site of Injection
				Slope/min (D)	Flow ml/100 g/min	Mean	
N.P.	40	3	27	0.160	25.8	28.6	fundus
				0.184	28.6		body
				0.198	31.5		isthmus
K.A.	33	1	23	0.107	17.3	16.6	body
				0.099	15.9		without
L.O.	23	0	10	0.098	15.7	15.0	body
				0.088	14.2		isthmus
E.A.	17	0	22	0.056	9.0	11.1	body
				0.051	8.2		isthmus
B.H.	24	2	pre-menstr	0.253	40.5	40.1	body
				0.247	39.7		isthmus
A.O.	18	0	5	0.167	26.8	23.4	fundus
				0.124	20.0		body
E.A.	30	4	14	0.200	32.2		body
S.O.	30	5	prolif phase	0.153	24.8		body
K.A.	24	0	18 <sup>7</sup>	0.082	13.2		body
M.A.	30	4	19	0.210	33.8		body
M.B.	23	2	7	0.104	16.8		body
Mean $\pm$ SE							23.0 $\pm$ 2.98

Isthmus here denotes the lower segment of the uterine body at the level of the vesico-uterine peritoneal reflection.

### Pregnant uteri

The results in the pregnant group are seen in Tables III, IV and V. The mean  $^{133}\text{Xe}$  myometrial clearance in 40 pregnant women regardless of the placental site was  $18.6 \pm 1.37$  ml/100 g/min. Again there was a wide range from 4.5 to 39.5 ml. If however the isotope was injected in the myometrium at the placental implantation site the mean clearance rate was  $25.8 \pm 1.67$  ml/100 g/min (range 19.3–39.5) and if it was made in non-placental myometrium (myometrium proper) the mean clearance rate was  $10.6 \pm 0.97$  ml/100 g/min (range 4.5–16.1).

It is obvious from table V that there was no difference in

26 1119 NF

FUNDUS

CORPUS

ISTHMUS

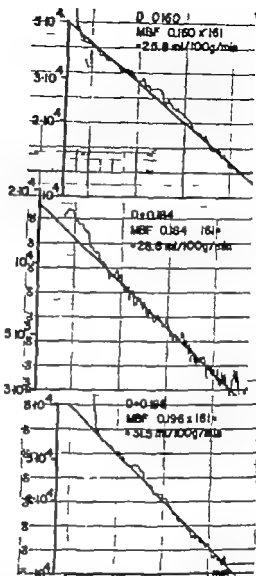


Fig. 2. Three recordings of myometrial  $^{133}\text{Xe}$  clearance in the same non-pregnant uterus. The figure illustrates the reproducibility of the clearance method and the fact that there is no essential difference in clearance rate between different parts of the non-pregnant uterus. The ordinates denote gamma-radiation in cpm.

clearance in 7 parous women was  $27.6 \text{ ml/100 g/min}$  as compared with  $15.1 \text{ ml}$  in 4 non parous women. The lowest recorded value  $8.2 \text{ ml/100 g/min}$ , was found in a 17 years old gravida 0 with a small juvenile uterus.

Table IV  $^{133}\text{Xe}$  Clearance in Myometrium in Late Pregnancy

Patient Name	Age Years	Pregnancy No.	Week of Pregnancy	$^{133}\text{Xe}$ Clearance		Site of Injection
				Slope/min (D)	Flow ml/100 g/min	
E.A.	21	1	25	0.129	19.5	plac. myometr
L.N.	19	1	26	0.083	13.4	not determined
I.L.	35	4	29	0.120	19.3	plac. myometr
			38	0.157	25.2	plac. myometr
L.A.	26	9	32	0.059	9.5	myometr proper
			35	0.090	15.8	myometr proper
B.H.	21	2	35	0.078	12.5	myometr proper
B.O.	24	1	37	0.059	8.7	myometr proper
K.E.	23	2	37	0.091	14.6	myometr proper
A.W.	24	1	37	0.243	39.0	plac. myometr
I.B.	31	3	37	0.140	22.6	plac. myometr
G.E.	27	2	38	0.089	14.3	not determined
K.A.	17	1	38	0.132	21.2	plac. myometr
I.H.	23	3	38	0.080	12.9	not determined
B.H.	20	1	39	0.090	14.5	myometr proper
A.A.	40	3	39	0.129	20.7	plac. myometr
R.R.	27	3	39	0.042	6.8	myometr proper
P.H.	25	3	39	0.051	8.2	myometr proper
M.B.	26	1	39	0.245	39.5	plac. myometr
M.L.	23	3	39	0.084	13.4	not determined
L.D.	25	1	40	0.100	16.1	myometr proper
I.K.	19	1	40	0.160	25.8	plac. myometr
M.J.	19	1	41	0.066	10.6	myometr proper
A.S.	21	2	41	0.160	25.8	not determined
E.L.L.	19	1	43	0.055	8.8	not determined
				0.061	11.3	not determined
Mean $\pm$ SE				17.6 $\pm$ 1.82		

g/min, i.e. to the level of myometrium proper. This coincided with a steep fall in the urinary oestriol excretion.

#### Puerperal uteri

The mean of 11 measurements of myometrial  $^{133}\text{Xe}$  clearance in the first few days post partum was  $32.1 \pm 3.99$  ml/100 g/min (Table VI). There were great individual variations in this group

Table III.  $^{133}\text{Xe}$  Clearance in Myometrium in Early and Midpregnancy

Patient	Age Years	Preg nancy No.	Week of Preg nancy	$^{133}\text{Xe}$ Clearance		Mean	Site of Injection
				Slope/mln (D)	Flow ml/100 g/min		
	39	4	10	0.028	4.5		myometr proper
	40	5	11	0.143	23.0		plac. myometr
	40	2	14	0.126	20.2	22.4	plac. myometr
				0.153	24.6		plac. myometr
	34	3	14	0.082	13.2		myometr proper
	31	5	14	0.218	35.0		plac. myometr
	37	3	14	0.150	24.2		plac. myometr
	35	4	15	0.091	14.6		plac. site not
				0.114	18.3	19.2	determined
				0.154	24.8		
	38	4	16	0.191	30.8		plac. myometr
	38	3	16	0.150	24.2		not determined
	26	6	17	0.163	26.2		not determined
	38	5	19	0.123	19.9		plac. myometr
	40	5	19	0.041	6.6		myometr proper
	24	2	20	0.147	23.6		not determined
	19	1	21	0.042	6.8		myometr proper
	36	10	22	0.163	26.3		plac. myometr
	38	7	22	0.127	20.4		plac. myometr
	38	4	23	0.077	12.4		myometr proper
Mean $\pm$ SE				19.9 $\pm$ 2.06			

myometrial  $^{133}\text{Xe}$  clearance between early and midpregnancy on one hand and late pregnancy on the other either with respect to placental or to non placental myometrium

The myometrial  $^{133}\text{Xe}$  clearance in a case of intrauterine foetal death is illustrated by Fig. 3. Two measurements in the same part of the uterine wall at 25 and 34 weeks of pregnancy in a diabetic woman gave the clearance values 22.4 and 33.4 ml/100 g/min, respectively. The injections had apparently been into the myometrium at the placental site although this was not proved by arteriography. In the 36th week of pregnancy signs of foetal death developed. The clearance examination was repeated at the same site and now the clearance rate had dropped to 9.4 ml/100



Table VI. <sup>133</sup>Xe Clearance in Myometrium in the Puerperium

Patient Name	Age Years	No. of Pregnancies	Days Post Partum	<sup>133</sup> Xe Clearance		Mode of Injection
				Slope/min (D)	Flow ml/100 g/min	
MB	28	9	0	0.121	19.5	transabdom.
GBD	22	2	1	0.235	37.8	transabdom.
UBA	20	1	1	0.164	26.4	transabdom.
AA	23	1	1	0.138	22.3	transabdom.
RL	32	5	2	0.267	43.0	transperit.
				0.240	38.6	laparotomy
CE	31	6	3	0.256	41.5	laparotomy
HL	41	1	3	0.080	12.9	transabdom.
HQ	38	5	3	0.360	58.0	transabdom.
MB	25	1	3	0.126	20.2	transabdom.
AR	38	4	4	0.208	33.4	transperit.
Mean $\pm$ SE				32.1 $\pm$ 3.99		

also. The highest recorded clearance rate, 58.0 ml/100 g/min, was found in a puerperal uterus on the third day after delivery

### Comparison between groups

Analysis of variance of the mean values of the four groups non-pregnant, early and midpregnant, late pregnant, and puerperal women is given in Table VII. It is clear that the null hypothesis that the four mean values are all equal can be rejected at the 1 per cent level of significance. Application of Scheffé's method of multiple comparisons shows that the mean myometrial <sup>133</sup>Xe clearance in the puerperium is significantly higher than that of both pregnant groups. The mean value in the non-pregnant group is not significantly different from any of the other groups. There is no statistically significant difference between the mean value in early and midpregnancy and that in late pregnancy.

A comparison between the <sup>133</sup>Xe clearance of myometrium proper, placental myometrium and non-defined myometrium (total series in table V) by the same methods (table VIII) shows that the differences between all three mean values are statistically significant ( $P < 0.01$ ).

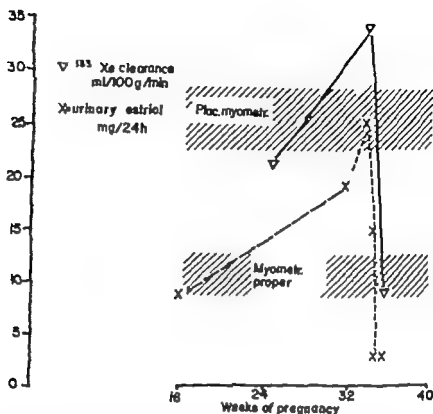


Fig. 3 Myometrial  $^{133}\text{Xe}$  clearance and urinary oestriol excretion in a case of intrauterine foetal death in a diabetic woman. The shadowed areas represent the mean  $^{133}\text{Xe}$  clearance  $\pm 2 \times$  standard error of the mean in placental and non-placental myometrium, respectively. The figures of the ordinate indicate both  $^{133}\text{Xe}$  clearance in ml/100 g/min and urinary oestriol excretion in mg/24 h.

Table V Mean Values of Myometrial  $^{133}\text{Xe}$  Clearance in Pregnancy in Relation to Gestation Period and Placental Site

	$^{133}\text{Xe}$ Clearance ml/100 g/min $\pm$ SE			
	Myometrium Proper	Placental Myometrium	Not Defined	Total
Early and midpregnancy	$8.7 \pm 1.73$ (5)	$25.3 \pm 1.86$ (8)	$23.3 \pm 1.48$ (4)	$19.9 \pm 2.08$ (17)
Late pregnancy	$11.6 \pm 1.08$ (9)	$26.3 \pm 2.90$ (8)	$15.0 \pm 2.24$ (6)	$17.6 \pm 1.82$ (23)
Total series	$10.6 \pm 0.97$ (14)	$25.8 \pm 1.67$ (16)	$18.3 \pm 1.95$ (10)	$18.6 \pm 1.37$ (40)

Figures in brackets indicate number of cases.

Table VIII

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	F	Critical Value $F_{.99}$
Between groups	1728.54	2	863.27	27.07	5.18
Within groups	1179.86	37	31.89		
Total	2908.40	39			

### Discussion

$^{135}\text{Xe}$  clearance is a measure of the capacity of the capillary blood flow to remove the injected isotope sometimes thought of as nutritional blood flow but better called effective capillary blood flow. It must be understood that the distribution of this capillary blood flow may vary independently of the total regional blood flow since its regulation seems to be especially dominated by local metabolic factors (*cf. e.g. Follow 1967*). Factors influencing the clearance rate then must include the degree of vascularity of the tissue as well as, to some extent at least, the number of capillaries open to blood flow at any given moment.

The clearance method of estimating tissue blood flow has been criticized because of the variability of the clearance rate depending on, for example the site of injection and the composition of the tissue in which the tracer is deposited (*Assali and Morris 1964*). In the present study one major source of variability was controlled when the relation between the injection site and the placental implantation site was taken into account. Another point is of importance. Judging from the mono-exponential elimination curves obtained from the non-pregnant uterus, myometrium is a rather homogenous tissue. Curves of a pronounced multi-exponential course suggests deposition in more than one type of tissue. Thus only those experiments where the clearance curve was close to mono-exponential were accepted for calculation of blood flow. The fairly good agreement between the results obtained at repeated observations in the same patient confirms the usefulness of the clearance method in this type of tissue.

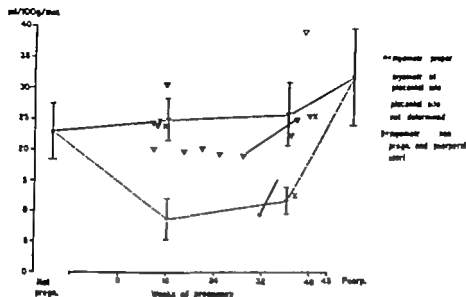


Fig. 4  $^{133}\text{Xe}$  clearance in non-pregnant, pregnant and puerperal myometrium. For non-pregnant and puerperal uteri only mean values are given. In the pregnant group open symbols represent individual values, filled symbols mean values. Vertical bars represent  $\pm 2 \times$  standard error of the mean. The figure illustrates the separation of myometrial  $^{133}\text{Xe}$  clearance in pregnancy at two different levels representing different areas of the uterine wall placental and non-placental myometrium.

Table VII

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares	f	Critical Value f .99
Between groups	1654.77	3	551.59	5.73	4.13
Within groups	5581.79	58	96.24		
Total	7236.56	61			

The results in non pregnant pregnant and puerperal women are collected in Fig. 4 illustrating the separation of myometrial  $^{133}\text{Xe}$  clearance during pregnancy into two different levels, representing different areas of the uterine wall, myometrium at the placental site and non-placental myometrium.

placental and non-placental myometrium persists throughout the pregnancy

This finding, however, is in contrast with the results of Moore and Myerscough (1957) who found the clearance rate of  $^{24}\text{Na}$  to be significantly lower at the placental implantation site than in non-placental myometrium. These authors were unable to explain this unexpected finding. It is possible that the fact that sodium is not freely diffusible may be responsible for this difference in results. Particularly interesting is the observation that progesterone blocks sodium transport through the cell membrane (Jung, 1965) in view of Caspo's theory (1959) that progesterone produced by the placenta diffuses directly into the neighbouring myometrium (cf Bengtsson 1967)

There is an increased vascularity at the placental implantation site but this may not be the sole explanation of the higher  $^{133}\text{Xe}$  clearance rate at this location. The case described (Fig. 3) where the clearance rate at the placental site dropped to the level of non-placental myometrium when the foetus died, illustrates this. Again, a local effect of placental hormones on the vascular bed may be responsible. The immediate fall in oestriol excretion coinciding with foetal death suggests that this hormone may be implicated. The vasodilating effect of oestrogens is well known. Greiss and Marston (1965) found that conjugated equine oestrogens caused a mean 36 per cent increase in uterine blood flow in pregnant ewes within two hours.

This effect of placenta on the local  $^{133}\text{Xe}$  clearance rate in the myometrium must of course have a decisive effect on results obtained by this method. In this selected series consisting of about equal numbers of observations on placental and non-placental myometrium, the average  $^{133}\text{Xe}$  clearance rate was 18.6 ml/100 g/min as compared with 12.7 ml in the series of Lysgaard and Lefèvre (1963). These authors did not localize the placenta and all their experiments were performed in late pregnancy where the chance of hitting non-placental myometrium must be proportionately greater. Thus, a low mean value is to be expected. The falling  $^{133}\text{Xe}$  clearance rate during the second half of pregnancy found by Gullhem *et al.* (1965) may well have the same explanation.

When compared with  $^{24}\text{Na}$  which has been used in most earlier studies (Brown and Veall 1953 Morris *et al* 1955 Johnson and Clayton 1957 Moore and Myerscough 1957 Dixon *et al* 1963)  $^{133}\text{Xe}$  has the advantage of being chemically inert and freely diffusible through the cell membranes. Its gaseous nature soft gamma radiation and short half life (5.3 days) makes it convenient to handle and an ideal radioactive test substance in man.

The average  $^{133}\text{Xe}$  clearance in myometrium of the non-pregnant uterus in the present series 23 ml/100 g/min, is the same as the mean value found by Munck *et al* (1964) with a transvaginal injection of the isotope in the isthmus uteri. Repeated observations in the same women showed no difference between the upper and lower segments of the uterine body. There was however a wide scatter of the individual values. The only factor found to influence this variability was parity. Although the group is too small to permit statistical analysis the observation that parous women had a myometrial  $^{133}\text{Xe}$  clearance rate twice that of non-parous women is noteworthy. By far the lowest clearance rate was found in a 17 year-old nulliparous girl with a small juvenile uterus. This is in agreement with the observation by Horn (1916) that the blood vessels of the parous uterus are larger and more abundant.

The results in pregnant women confirm the finding by Falk *et al* (1967) that the myometrial  $^{133}\text{Xe}$  clearance at the placental site is about twice that of non placental myometrium. Falk *et al*. made their experiments only in midpregnancy and calculated placental myometrial blood flow from the first rapid phase of elimination curves obtained in the vicinity of placenta and blood flow in myometrium proper both from the slow phase of these curves and from mono-exponential curves obtained at a distance from placenta. The values found, however about 9 ml/100 g/min for myometrium proper and 21.8 ml/100 g/min for placental myometrium, are in close agreement with the mean values in early and midpregnancy in the present study 8.7 and 24.9 ml/100 g/min respectively. In addition measurements in late pregnancy revealed approximately the same clearance rates 11.6 and 26.3 ml/100 g/min respectively showing that this difference between

between 500 and 700 ml/min is obtained (Assali *et al.* 1953 Metcalfe *et al.* 1955). In view of the considerations given above these figures may be too low. If a similar procedure is performed with the observed <sup>133</sup>Xe clearance values they should be multiplied only by the weight of the uterus, which is about 1 kg at term. The total myometrial blood flow at term then may be approximately 150–200 ml/min. The major part of the total uterine blood flow is destined for the spiral arteries, emptying their blood into the intervillous spaces. This fraction cannot be estimated by the clearance method, as there seems to be no means of puncturing the intervillous space with certainty (Fuchs, Speckman and Assali 1963).

As pointed out earlier <sup>133</sup>Xe clearance reflects the effective capillary blood flow which is regulated by the local metabolic needs of the tissue. From this point of view the high <sup>133</sup>Xe clearance in puerperal myometrium is interesting. The mean value of 32.1 ml/100 g/min is in sharp contrast with the mean uterine blood flow found by Assali *et al.* (1953) using the nitrous oxide method, 9 ml/100 g/min. It is hard to explain this great difference as the figures should be comparable in the absence of foetus and placenta. The figure of Assali, however, is based on 4 observations, 3 of which were immediately after caesarean section. There is reason to believe that the increased uterine tone immediately post partum diminishes the blood flow considerably. The 11 observations in the present series were made 1–4 days after delivery on the intact uterus. The high clearance rate found may be explained by an increased capillary blood flow demanded by the intense metabolic processes involved in puerperal uterine involution.

## SUMMARY

1. <sup>133</sup>Xe clearance in the myometrium was studied in 11 non-pregnant women, in 40 pregnant women between the 10th and 43rd weeks of gestation, and in 10 women in the first few days of the puerperium. A total of 75 measurements were performed.
2. The mean <sup>133</sup>Xe clearance in non-pregnant myometrium was 23.0 ml/100 g/min. The wide scatter found (8.2–40.5 ml) could

In the present series there was no demonstrable difference in myometrial  $^{133}\text{Xe}$  clearance at different periods of gestation, either at the placental site or in non placental myometrium. This is in agreement with the observation by Assali *et al.* (1960) who compiled their own results obtained by the nitrous oxide method with those of Metcalfe *et al.* (1955) and found that the blood flow per 100 g of pregnant uterus does not change significantly throughout the pregnancy. Assali *et al.* (1953 1960) found a mean uterine blood flow at midpregnancy of 8.9 ml/100 g/min and 15 ml at term. The average value of Metcalfe *et al.* (1955) was 12.4 ml/100 g/min at term. These values cannot be compared with those obtained with the  $^{133}\text{Xe}$  method as they constitute a measure of total uterine blood flow calculated per 100 g of uterus, placenta and foetus. They must also be regarded as minimal values.

Before the present investigation was started a pilot study on 8 cases of legal abortion and sterilization showed that the  $^{133}\text{Xe}$  clearance in myometrium was greatly influenced by exposure of the pregnant uterus in the laparotomy wound. The first injection immediately after the abdomen was opened often gave a clearance rate around 15–25 ml/100 g/min. When the examination was repeated 20–30 minutes later figures as low as 4–5 ml/100 g/min were obtained from the same area. This coincided with evidence of increased myometrial tone such as hardening and blanching of the uterine wall. This preliminary observation was in fact the reason for the technique used in the present study with transperitoneal injection of the isotope without exposing the uterus in the laparotomy wound. That uterine contractions reduce uterine blood flow is well known. Ahlquist (1950) found a decrease in uterine blood flow of 10–50 per cent with a rise in the uterine pressure of 20–30 mm Hg in dogs and Borell Fernström, Ohlson and Wikvist (1965) demonstrated with a cineangiographic technique that in women there is a slowing of the uterine blood flow with contractions. They attributed this finding to local compression of the arterioles by the myometrium.

If the values mentioned above calculated by using the nitrous oxide method are multiplied by an estimated weight of uterus and its contents of 4.5 kg, a total uterine blood flow at term of



- Csapo, A. *Ann. N.Y. Acad. Sci.* 75 790 1959
- Dixon, H. G., Broune J. C., McC., and Davey M. A., *Lancet* II 369 1963
- Falk, V., Forlomen B., and Lindell S. E., *Strahlentherapie (Sonderb.)* 65 162, 1967
- Folchow B. *Triangle* 8, 70 1967
- Fuchs, F., Spackman, T. and Assali, N. S. *Amer. J. Obstet. Gynec.* 86 226 1963
- Gresta, F. C., and Marston, E. L., *Amer. J. Obstet. Gynec.* 93 720 1965
- Guthrie, P., Poutonier A., and Poutonier G. *Gynec. Obstet. (Paris)* 64 313, 1965
- Horn, O. *Histologiske studier over den menneskelige uterus* Dins. København, 1916
- Huckabee W. E., *Amer. J. Obstet. Gynec.* 84 1623 1962
- Hynes, F. B. and Leitch L., *The Physiology of Human Pregnancy* Blackwell Scientific Publ., Oxford, 1964
- Johnson, T. and Clayton, C. G., *Brit. Med. J.* 1 312, 1957
- Jung, H. *Zur Physiologie und Klinik der hormonalen Uterusregulation*, Bibliotheca Gynecologica 33 1963
- Kelly S. S., *Amer. Heart J.* 38, 321 1949
- Kjellmer L., Lindberg, L., Pitarovsky L., and Tönnestam, H., *Acta Physiol. Scand.* 89 69 1967
- Lazars, N. A., *Strahlentherapie (Sonderb.)* 60 37 1965
- Lazars, N. A., Lindberg, L., and Mianck, O. *Lancet* I 688, 1964
- Lyrgaard, H. and Lefèvre H., *Acta Obstet. Gynec. Scand.* 44 401 1965
- Mercelf J., Ramsey S. L., Ramsey L. H., Reid, D. E., and Barwell C. S., *J. Clin. Invest.* 34 1632, 1955
- Moore P. T. and Myerrough P. R. *J. Obstet. Gynec. Brit. Emp.* 111 207 1957
- Morris, N., Osborn, S. B. and Payling-Wright H. *Lancet* I 323, 1955
- Mianck, O., Lyrgaard, H., Poutonier G., Lefèvre H., and Lazars, N. A. *Lancet* I 1421 1964
- Spetz S. and Jansson I. *Acta Obstet. Gynec. Scand.*, 48 285 1969
- Toussaint K. H. *Scand. J. clin. Lab. Invest.* 21 65 1968

Received on Sept. 5 1968

in part be explained by the differences in blood flow between parous and non parous uteri. There was no difference between different parts of the uterine body.

3 The mean myometrial  $^{133}\text{Xe}$  clearance in pregnant uteri was 18.8 ml/100 g/min. The mean clearance rate in the myometrium at the placental implantation site was 25.8 ml/100 g/min and in non-placental myometrium 10.6 ml/100 g/min. This difference is statistically significant.

4 There was no difference in  $^{133}\text{Xe}$  clearance rate between early and midpregnancy on one hand and late pregnancy on the other either with respect to placental or to non placental myometrium. This suggests that the effective capillary blood flow in the myometrium per unit weight of tissue is unchanged throughout the pregnancy.

5 The highest mean  $^{133}\text{Xe}$  myometrial clearance rate, 32.1 ml/100 g/min was found in the puerperium.

### *Acknowledgements*

This study was aided by grants from the Medical Faculty of the University of Göteborg, the Swedish Medical Research Council and the Medical Society of Göteborg. The statistical analysis was performed by Mrs Gull Britt Palm fil. kand.

### REFERENCES

- Ahlquist R. P. *J Amer Pharm. Ass. (Scient. Ed.)* 39: 370, 1950  
 Andersen A. M. and Ladefoged, J. *Scand J clin. Lab. Invest.* 19: 72, 1967  
 Assali N. S., Douglass R. A., Baird W. W., Nicholson D. B., and Szymoro R. *Amer J Obstet. Gynec.* 66: 748, 1953  
 Assali N. S. and Morris J. A. *Obstet. Gynec. Surv.* 19: 923, 1964  
 Assali N. S., Rauramo L. and Peltonen, T. *Amer J Obstet. Gynec.* 79: 86, 1960  
 Bengtsson L. Ph. *Progesterone and the Myometrium in Human Pregnancy*  
*Adv. Obstet. Gynec.*, Ed. Marcus & Marcus, Baltimore, 1967  
 Borell U., Fernstöm, I., Ohlsson, L. and Wijkvist N. *Amer J Obstet. Gynec.* 93: 44, 1965  
 Browne J. C. McC. and Veall N. *J Obstet. Gynec. Brit. Emp.* 60: 142, 1953  
 Brownlee A. A. *Statistical Theory and Methodology in Science and Engineering*, New York, 1963

creased gradually suggesting constant resting muscle blood flow and rising skin blood flow during the second half of pregnancy. In the present investigation the peripheral circulation has been studied with the same techniques in a group of pregnant diabetic women to detect possible deviations from normal pregnancy.

$^{133}\text{Xe}$  clearance has been shown to be a useful tool in studies of myometrial circulation during pregnancy (Lysgaard and Løffeire 1965, Guillemin *et al.* 1965, Falk *et al.* 1967, Jansson 1969). With this technique it was demonstrated that the myometrial blood flow at the placental bed is about twice that of non-placental myometrium (Falk *et al.* 1967, Jansson 1969) and that the myometrial blood flow per unit weight of tissue is largely unchanged throughout pregnancy (Jansson 1969). Thus, another purpose of this investigation was to study the  $^{133}\text{Xe}$  clearance of the myometrium in diabetic pregnancy and compare the results with those previously obtained in normal pregnancy.

### Material

Twenty-one pregnant women with diabetes mellitus were studied. Relevant clinical data on each patient are given in Table I. The severity of the diabetes was classified according to White (1949) with the modifications used by Pedersen (1967): i.e. Class A diabetes treated by diet alone, Class B insulin treated diabetes diagnosed at the age of 20 or later and without late diabetic complications, Class C diabetes diagnosed before 20 years of age and without late diabetic complications, Class D diabetes with non-proliferative retinopathy, Class F diabetes with vascular nephropathy and/or proliferative retinopathy. Class E (calcification of pelvic arteries) was not used and there were no A- and F-cases in the series studied. Prognostically bad signs during the present pregnancy (PBSP) according to Pedersen and Pedersen (1965) are also noted in the table. The patients were treated according to the now widely accepted principles with repeated admissions to hospital and an attempt throughout pregnancy to keep the blood glucose level as close to normal as possible to avoid keto-acidosis. The time for and the mode of delivery was chosen in each individual case on the basis of the severity of the

## FOREARM AND MYOMETRIAL BLOOD FLOW IN DIABETIC PREGNANCY STUDIED BY VENOUS OCCLUSION PLETHYSMOGRAPHY AND $^{133}\text{XENON}$ CLEARANCE

BY

INGE JANSSON

When pregnancy is complicated by diabetes mellitus the outcome of the pregnancy is decided to a great extent by the degree of maternal vascular disease (*cf e.g. White 1949 1958 Pedersen 1967*). That the state of the vessels may interfere with the normally occurring circulatory changes during pregnancy is obvious. In spite of this fact very few studies of the circulation in diabetic pregnancy have been performed.

Spetz (1965) studied the peripheral circulation in diabetic pregnancy by means of venous occlusion plethysmography of the forearm and found a progressive increase of the same magnitude as during normal pregnancy. Brudenell, Miles and Coleman (1961) used the clearance rate of radioactive sodium ( $^{24}\text{Na}$ ) from the uterine wall as an indicator of myometrial blood flow in diabetic pregnancy but were not able to demonstrate any difference from normal pregnancy.

$^{133}\text{Xenon}$  has certain advantages over  $^{24}\text{Na}$  in measurements of local tissue blood flow (Lassen *et al.* 1964) and can be used to evaluate the blood flow selectively in different tissue compartments. Sper and Jansson (1969) found the  $^{133}\text{Xe}$  clearance in forearm muscle to be unchanged during normal pregnancy while the forearm blood flow recorded by the plethysmograph in-

Table I. Continued

Age Years	Preg- nancy No.	White Class	IBCF	Hydram- nios	Week of Delivery	Mode of Delivery	Infant
19	1	C	0	0	39	Induction + VE	♂ 3310 g cong. heart disease?
22	1	B	0	0	40	Induction + VE	♂ 3390 g normal
22	1	B	0	0	40	N.D	♂ 4180 g normal

breastmilk

ISP prognostically bad signs in pregnancy (Pedersen and Pedersen, 1965) toxemia, keto-acidosis, neglectors: 1 patients neglecting proper diabetic control during pregnancy pyelo-nephritis.

D normal delivery in OA position. VE = vacuum extraction.

IDS idiopathic respiratory distress syndrome.

disease, the obstetrical history the course of the pregnancy and daily determinations of urinary oestriol excretion.

### Methods

To obtain best possible basal conditions the patients rested in bed for about 30 minutes before any measurements were made. The room temperature was maintained at 22 °C. The  $^{133}\text{Xe}$  muscle clearance measurement was always performed before the plethysmographic examination. Both methods of estimating peripheral blood flow were employed 2-4 times during the course of the pregnancy. The results were compared with those previously obtained in healthy non-pregnant and pregnant women (Spetz and Jansson 1969).

1.  $^{133}\text{Xe}$  clearance in forearm muscle. The isotope was injected into the extensor musculature of the forearm and the clearance rate recorded as described in detail previously (Spetz and Jansson 1969). The local muscle blood flow (MBF) was calculated from the slope of the elimination curve (D) according to the

Table I. *Clinical Data*

Case No.	Age Years	Pregnancy No.	White s Class	PBSP	Hydramnios	Week of Delivery	Mode of Delivery	Infant
1	18	1	C	tox.	0	37	Induction + VE	♂ 3700 g neonat. death
2	25	1	C	0	0	38	Induction + N.D	♀ 2400 g normal
3	36	4	B	acidosis negl	0	35	N.D	♀ 3320 g dysmature
4	27	1	B	0	0	37	Induction + VE	♀ 3000 g neonat. death
5	20	1	C	0	+	36	N.D	♀ 2950 g normal
6	23	4	D	0	0	38	Caesarean section	♀ 2640 g normal
7	29	1	D	tox.	0	37	N.D	♂ 2930 g Intrauterine death
8	24	2	C	0	0	38	Induction + N.D	♂ 3970 g normal
9	21	1	C	0	0	36	Caesarean section	♂ 3780 g normal
10	27	5	C	acidosis	0	33	N.D	♂ 1730 g multiple malform.
11	26	1	B	0	+	37	Caesarean section	♂ 2850 g normal
12	35	2	D	0	+	38	Caesarean section	♀ 3410 g normal
13	18	2	D	0	+	39	Caesarean section	♂ 2550 g dysmature
14	23	1	C	0	0	39	Induction + N.D	♀ 2580 g normal
15	31	2	C	0	+	38	Induction + N.D	♂ 4530 g dead at 3 m. brain tumor
16	28	2	D	0	0	38	Caesarean section	♀ 3790 g normal
17	23	1	D	0	0	39	Caesarean section	♂ 3400 g normal
18	19	1	C	pyelo-neph	++	38	Spont. breech	♂ 3940 g normal

Table I. Continued

Age Years	Preg- nancy No.	White's Class	PBCP	Hydram- nios	Week of Delivery	Mode of Delivery	Infant
19	1	C	0	0	39	Induction + VE	♂ 3310 g cong. heart disease?
22	1	B	0	0	40	Induction + VE	♂ 3390 g normal
22	1	B	0	0	40	N.D.	♂ 4180 g normal

Abbreviations:

SP: prognostically bad signs in pregnancy (Pedersen and Pedersen, 1965): toxemia, keto-acidosis, neglectors i.e. patients neglecting proper diabetic control during pregnancy ptero-nephritis.

D: normal delivery in OA position. VE = vacuum extraction.

DS: idiopathic respiratory distress syndrome.

disease the obstetrical history the course of the pregnancy and daily determinations of urinary oestriol excretion.

### Methods

To obtain best possible basal conditions the patients rested in bed for about 30 minutes before any measurements were made. The room temperature was maintained at 22°C. The  $^{133}\text{Xe}$  muscle clearance measurement was always performed before the plethysmographic examination. Both methods of estimating peripheral blood flow were employed 2-4 times during the course of the pregnancy. The results were compared with those previously obtained in healthy non-pregnant and pregnant women (Spetz and Jansson 1969).

1.  $^{133}\text{Xe}$  clearance in forearm muscle: The isotope was injected into the extensor musculature of the forearm and the clearance rate recorded as described in detail previously (Spetz and Jansson 1969). The local muscle blood flow (MBF) was calculated from the slope of the elimination curve (D) according to the

equation  $MBF = D/161$  ml per 100 g of tissue per minute (Lassen *et al.* 1964)

2. *Plethysmography* The blood flow in the right forearm was measured by venous occlusion plethysmography in the same manner as described by Spector (1964). The water temperature of the plethysmograph was always maintained at 34 °C. The mean of at least ten measurements was taken as the total forearm blood flow at rest, expressed in ml per 100 ml of tissue per minute.

3. *<sup>133</sup>Xe clearance in myometrium.* The technique used has been described in detail before (Jansson 1969). In most cases the <sup>133</sup>Xe solution was injected transabdominally into the anterior uterine wall without knowledge of the placental site. In two patients (no 13 and 17) the placental site was determined at Caesarean section. In one (10) by pelvic arteriography and in another one (14) by isotope (<sup>99m</sup>Tc) scanning. In four additional cases (12, 18, 19, 20) the placenta could be located with the aid of a Doptone ultrasonic foetal pulse detector (Smith Kline Instrument Co., England). Bishop (1966) has shown that the placental site can be determined with this instrument in about 85 per cent of cases. Whenever possible the <sup>133</sup>Xe clearance examination was repeated at varying intervals during the pregnancy and always in approximately the same area of the uterine wall after allowing for the uterine growth. As the myometrial blood flow per unit tissue is largely unchanged throughout pregnancy a mean value was calculated for those patients where more than one examination was made.

*Statistics.* Mean values, standard error of the mean (SE) and regression lines were calculated by conventional statistical methods. In the text and tables mean values are given  $\pm$  SE. The significance of differences between groups was tested by the *t* test. With unequal variances the Welch procedure was applied.

The reproducibility of the <sup>133</sup>Xe clearance method when applied to myometrium was tested by analysis of variance showing a significantly smaller variation ( $P < 0.001$ ) in the same patient than between patients. Furthermore a test of squared differences between the first and second measurements showed that the difference between two measurements in the same patient was not significantly different from zero.



The statistical formulas used were taken from Brownlee (1965). When not otherwise stated the tests of significance of differences were performed at the 5 per cent level.

### Results

#### Peripheral blood flow

The results of 23 determinations of forearm blood flow by plethysmography and 30 measurements of  $^{133}\text{Xe}$  muscle clearance in 16 pregnant diabetics are given in Table II. Five of these cases were examined only once the rest two to four times during the course of the pregnancy. The mean total forearm blood flow at rest for the whole group of pregnant diabetic women,  $3.9 \pm 0.48$  ml/100 ml/min, is significantly higher than the mean resting forearm blood flow of non-pregnant healthy women,  $2.5 \pm 0.32$  ml/100 ml/min. The mean  $^{133}\text{Xe}$  clearance in forearm muscle in the pregnant diabetics,  $3.3 \pm 0.25$  ml/100 g/min, however is not significantly different from that of normal non-pregnant women  $3.2 \pm 0.22$  ml/100 g/min nor from that of normal pregnant women  $3.6 \pm 0.24$  ml/100 g/min.

The mean values for total forearm blood flow and  $^{133}\text{Xe}$  muscle clearance at different stages of pregnancy are given in Table III. The mean total forearm blood flow in the period 13-24 weeks of pregnancy  $2.9 \pm 0.50$  ml/100 ml/min, is not significantly different from that of non-pregnant healthy subjects. The differences between the mean values of forearm blood flow in the periods 25-32 and 33-37 weeks of gestation,  $3.8 \pm 0.71$  and  $5.8 \pm 0.99$  ml/100 ml/min, respectively and that of the non-pregnant group, however are statistically significant. None of the mean values of  $^{133}\text{Xe}$  muscle clearance in the diabetics at the different stages of pregnancy is significantly different from that in non-pregnant women.

Fig. 1 illustrates that the total forearm blood flow increased progressively from midpregnancy until 37 weeks while the forearm muscle blood flow as judged from  $^{133}\text{Xe}$  clearance remained unchanged throughout the pregnancy. As the diabetic pregnancies were interrupted before term no values were obtained after 37 weeks.

In Fig. 2 the individual total forearm blood flow values are

Table II. Forearm Blood Flow as Determined by Venous Occlusion Plethysmography and by  $^{133}\text{Xe}$  Muscle Clearance in Pregnancy Complicated by Diabetes Mellitus

Case No.	Week of Pregnancy	Blood Flow Plethysm. ml/100 ml/min $\pm$ SE	$^{133}\text{Xe}$ Clearance	
			Slope/min (D)	Flow ml/100 g/min
1	28		0.018	2.9
	32	$1.6 \pm 0.04$	0.010	1.6
	37	$1.8 \pm 0.07$	0.016	2.6
2	24		0.010	1.6
	31	$4.6 \pm 0.16$	0.016	2.6
	35	$3.7 \pm 0.16$	0.021	3.4
	37	$8.5 \pm 0.22$	0.049	7.8
3	34	$3.7 \pm 0.12$	0.012	2.0
4	32		0.021	3.4
5	31	$2.0 \pm 0.07$	0.017	2.7
	35	$4.8 \pm 0.19$	0.023	3.7
6	20	$1.6 \pm 0.05$	0.025	4.0
	21	$2.7 \pm 0.06$	0.027	4.4
	31	$5.9 \pm 0.35$	0.036	5.8
	35	$9.5 \pm 0.36$	0.026	4.2
	37		0.018	2.9
7	25	$2.6 \pm 0.16$	0.015	2.4
	34	$7.7 \pm 0.36$	0.036	5.8
8	20	$4.3 \pm 0.14$	0.023	3.7
	31	$7.4 \pm 0.37$	0.019	3.3
	34	$7.3 \pm 0.28$	0.023	3.7
9	22	$2.8 \pm 0.18$	0.018	2.9
	31	$3.4 \pm 0.26$	0.024	3.9
10	29	$2.3 \pm 0.09$	0.023	3.7
	32	$4.0 \pm 0.16$	0.029	4.7
11	18	$2.7 \pm 0.06$		
	23	$1.7 \pm 0.04$		
12	13		0.020	3.2
13	36		0.012	1.9
15	35		0.015	2.4
16	14		0.016	2.6
21	26		0.034	5.5
Mean $\pm$ SE		$3.9 \pm 0.48$		$3.3 \pm 0.25$

The group mean values are calculated on mean values for each case.

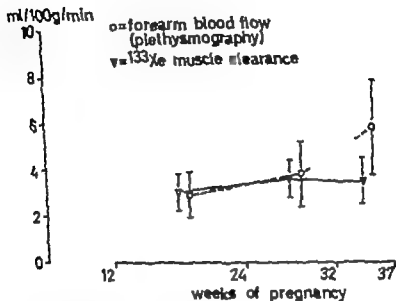


Fig. 1 Forearm blood flow determined by plethysmography and  $^{133}\text{Xe}$  clearance in forearm muscle at different stages of pregnancy complicated by diabetes mellitus. Mean values are given  $\pm$  SE.

Table III Forearm Blood Flow as Determined by Plethysmography and  $^{133}\text{Xe}$  Muscle Clearance at Different Stages of Pregnancy in Women with Diabetes Mellitus

Mean Values $\pm$ SE	— Number of Cases.		
Method	Weeks of Pregnancy		
	13-24	25-32	33-37
Plethysmography	2.9 $\pm$ 0.50	3.8 $\pm$ 0.71	5.8 $\pm$ 0.99
ml/100 ml/min	n = 4	n = 8	n = 7
$^{133}\text{Xe}$ clearance	3.0 $\pm$ 0.37	3.6 $\pm$ 0.39	3.5 $\pm$ 0.48
ml/100 g/min	n = 6	n = 10	n = 9

Table II. Forearm Blood Flow as Determined by Venous Occlusion Plethysmography and by  $^{133}\text{Xe}$  Muscle Clearance in Pregnancy Complicated by Diabetes Mellitus

Case No.	Week of Pregnancy	Blood Flow Plethysm. ml/100 ml/min $\pm$ SE	$^{133}\text{Xe}$ Clearance	
			Slope/min (D)	Flow ml/100 g/min
1	28		0.018	2.9
	32	$1.6 \pm 0.04$	0.010	1.6
	37	$1.8 \pm 0.07$	0.016	2.6
2	24		0.010	1.6
	31	$4.6 \pm 0.16$	0.016	2.6
	35	$3.7 \pm 0.16$	0.021	3.4
	37	$8.5 \pm 0.22$	0.049	7.8
3	34	$3.7 \pm 0.12$	0.012	2.0
4	32		0.021	3.4
5	31	$2.0 \pm 0.07$	0.017	2.7
	35	$4.8 \pm 0.19$	0.023	3.7
6	20	$1.6 \pm 0.05$	0.025	4.0
	21	$2.7 \pm 0.06$	0.027	4.4
	31	$5.9 \pm 0.35$	0.036	5.8
	35	$9.5 \pm 0.36$	0.026	4.2
	37		0.018	2.9
7	25	$2.6 \pm 0.16$	0.015	2.4
	34	$7.7 \pm 0.36$	0.036	5.8
8	20	$4.3 \pm 0.14$	0.023	3.7
	31	$7.4 \pm 0.37$	0.019	3.3
	34	$7.3 \pm 0.28$	0.023	3.7
9	22	$2.8 \pm 0.18$	0.018	2.9
	31	$3.4 \pm 0.26$	0.024	3.9
10	29	$2.3 \pm 0.09$	0.023	3.7
	32	$4.0 \pm 0.16$	0.029	4.7
11	18	$2.7 \pm 0.06$		
	23	$1.7 \pm 0.04$		
12	13		0.020	3.2
13	36		0.012	1.9
15	35		0.015	2.4
16	14		0.016	2.6
21	26		0.034	5.5
Mean $\pm$ SE		$3.9 \pm 0.48$		$3.3 \pm 0.25$

The group mean values are calculated on mean values for each case

Table IV  $^{133}\text{Xe}$  Clearance in Myometrium in Pregnancy Complicated by Diabetes Mellitus

Diabetes Mellitus					
Case No.	Week of Pregnancy	$^{133}\text{Xe}$ Clearance			Site of Injection
		Slope/min (D)	Flow ml/100 g/min	Mean	
1	28	0.042	6.8	9.5	Not defined
	32	0.055	8.9		
	37	0.060	12.9		
2	24	0.168	27.0	27.3	Not defined
	31	0.152	24.4		
	37	0.190	30.6		
3	34	0.090	14.5	30.3	Not defined
4	32	0.094	15.1		Not defined
5	31	0.089	14.1		Not defined
6	20	0.218	35.0	30.3	Not defined
	31	0.192	30.9		
	37	0.155	25.0		
7	25	0.129	21.2	27.5	Not defined
	34	0.210	33.8		
	36	0.057	9.2		
8	20	0.142	22.8	21.8	Not defined
	31	0.124	20.0		
	34	0.140	22.6		
9	22	0.115	18.5	21.5	Not defined
	31	0.152	24.4		
10	29	0.226	36.4	33.5	Placental myometr
	32	0.100	30.6		
12	33	0.182	29.3	30.8	Placental myometr
	36	0.200	32.2		
13	36	0.113	18.2	24.4	Placental myometr
14	36	0.110	17.7		Placental myometr
17	39	0.090	14.5		Non-placental myometr
18	33	0.060	9.7	24.4	Non-placental myometr
19	32	0.155	25.0		Placental myometr
	35	0.127	20.5		
	38	0.172	27.7		
20	31	0.147	23.6	21.1	Placental myometr
	35	0.115	18.5		
21	32	0.058	8.3	13.9	Not defined
	37	0.114	18.4		
Mean $\pm$ SE				20.3 $\pm$ 1.73	

This value was obtained after intrauterine death of the foetus and is not included in the mean value

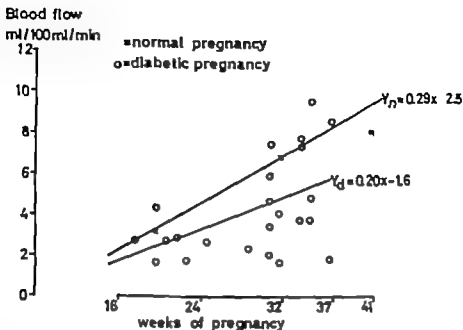


Fig. 2. Relation of forearm blood flow determined by plethysmograph to duration of pregnancy in normal pregnancy and in pregnancy complicated by diabetes mellitus.

plotted against the duration of pregnancy. For comparison the blood flow values for normal pregnant women where the same plethysmographic water temperature (34°C) was used, are given. The calculated regression lines for diabetic pregnancy between 16 and 37 weeks  $Y_d = 4.2 + 0.20(x - 29)$  and for normal pregnancy between 16 and 41 weeks  $Y_n = 6.8 + 0.29(x - 32)$  show that the forearm blood flow increased in a linear manner during the time intervals studied. The regression coefficients are not significantly different from each other but the lines are not identical ( $P < 0.05$ ). This means that the total forearm blood flow in the group of pregnant diabetic women increased at the same rate as in normal pregnancy but that the mean forearm blood flow was significantly lower in the pregnant diabetics.

#### <sup>133</sup>Xe clearance in myometrium

Thirty five technically acceptable measurements were performed in 18 diabetic patients at different periods of gestation (Table IV). The mean <sup>133</sup>Xe clearance in myometrium regardless of the

Table IV  $^{133}\text{Xe}$  Clearance in Myometrium in Pregnancy Complicated by Diabetes Mellitus

Case No.	Week of Preg nancy	$^{133}\text{Xe}$ Clearance			Site of Injection
		Slope/min (D)	Flow ml/100 g/min	Mean	
1	28	0.042	6.8	9.5	Not defined
	32	0.055	8.9		
	37	0.080	12.9		
2	24	0.168	27.0	27.3	Not defined
	31	0.152	24.4		
	37	0.190	30.6		
3	34	0.090	14.5	30.3	Not defined
4	32	0.094	15.1		Not defined
5	31	0.088	14.1		Not defined
6	20	0.218	35.0	27.5	Not defined
	31	0.192	30.9		Not defined
	37	0.155	25.0		Not defined
7	25	0.129	21.2	21.8	Not defined
	34	0.210	33.8		
	36	0.057	9.2		
8	20	0.142	22.8	21.5	Not defined
	31	0.124	20.0		
	34	0.140	22.6		
9	22	0.115	18.5	33.5	Placental myometr
	31	0.152	24.4		
	32	0.226	36.4		
10	32	0.190	30.6	30.8	Placental myometr.
	33	0.182	29.3		
	36	0.200	32.2		
13	36	0.113	18.2	24.4	Placental myometr
14	36	0.110	17.7		Placental myometr
17	39	0.090	14.5		Non-placental myometr
18	33	0.060	9.7	21.1	Non-placental myometr
19	32	0.155	25.0		Placental myometr.
	35	0.127	20.5		Placental myometr.
	38	0.172	27.7		Placental myometr.
20	31	0.147	23.6	13.9	Not defined
	35	0.115	18.5		
	37	0.058	9.3		
21	32	0.114	18.4	20.3 $\pm$ 1.73	Mean $\pm$ St.
	37	0.114	18.4		

This value was obtained after intrauterine death of the foetus and is not included in the mean value

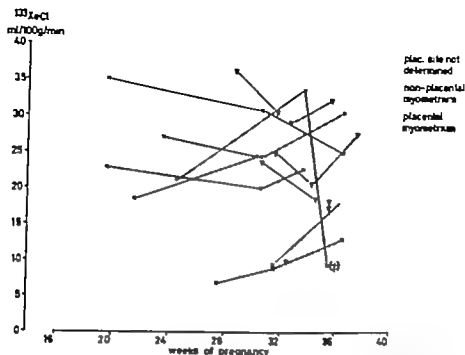


Fig. 3.  $^{133}\text{Xe}$  clearance in myometrium in pregnancy complicated by diabetes mellitus. The lines connect clearance values obtained at different stages of pregnancy in the same patients. The shadowed areas represent mean values of  $^{133}\text{Xe}$  clearance in placental and non-placental myometrium in normal pregnancy  $\pm 3$  SE. The value marked with a cross was obtained after intrauterine death of the foetus.

placental site was  $20.3 \pm 1.73$  ml/100 g/min. This is not significantly different from the corresponding mean value of normal pregnancy  $18.6 \pm 1.37$  ml/100 g/min. The mean  $^{133}\text{Xe}$  clearance in six cases where the  $^{133}\text{Xe}$  injection was made at the placental implantation site was  $24.3 \pm 2.69$  ml/100 g/min. The corresponding mean value in normal pregnancy was  $25.8 \pm 1.67$  ml/100 g/min. The difference is not statistically significant. Only in two cases could it be determined that the  $^{133}\text{Xe}$  injection was made in non-placental myometrium. The mean value 12.1 ml/100 g/min, is about equal to the corresponding mean  $^{133}\text{Xe}$  clearance in normal pregnancy 10.6 ml/100 g/min.

It is rather obvious from the graphic representation of the individual  $^{133}\text{Xe}$  myometrial clearance values in Fig. 3 that there was



no essential difference between different stages of pregnancy. The figure also illustrates the tendency of the clearance values to fall into two different groups representing placental and non-placental myometrium. The steep fall in  $^{133}\text{Xe}$  clearance after intrauterine death of the foetus in Case no. 7 has been commented on earlier (Jansson 1969).

No difference in the myometrial  $^{133}\text{Xe}$  clearance rate between cases with severe and those with mild diabetes or those with and without PBSP could be detected, nor was there any appreciable difference between cases with and without hydramnios.

### Discussion

Diabetes mellitus is characterized by early and frequent occurrence of vascular lesions, which are of two types: atherosclerosis which is especially prone to involve small vessels and the so called microangiopathy distinguished by thickening of the capillary basement membrane (for references see Warren, LeCompte and Legg, 1966). The lesions have been demonstrated in skeletal muscle as well as in the skin.

In non-pregnant diabetics a decreased peripheral circulation, especially in the skin of the toes and fingers has been observed with various techniques (Mendlowitz, Grossman and Alpert 1953; Megibow, Megibow, Pollack, Bookman and Osseman 1953; Bárdy 1955; Sigroth 1957; Weber and Wicht 1962) in a combined plethysmographic and histopathological study were able to correlate the abnormal distal circulation in diabetics with the occurrence of the above mentioned vascular lesions in the skin.

When diabetes is complicated by pregnancy the increasing peripheral blood flow will in some patients take place in a damaged vascular bed. In the present study it was shown that the peripheral blood flow measured by forearm plethysmography increased from an average of 2.9 ml in the period 16–24 weeks of pregnancy to 5.8 ml/100 ml/min at 33–37 weeks as compared to 3.5 and 7.6 ml/100 ml/min, respectively in normal pregnancy. The forearm blood flow increased at the same rate in the pregnant diabetics as in normal pregnancy but their mean forearm

blood flow was significantly lower. As can be seen from Fig. 2, however, there was a wide scatter of the individual values, some falling within the normal range. This is in agreement with the fact that only some of the patients in the age group studied may be expected to show vascular lesions severe enough to influence the rate of blood flow. The low values were often recorded in cases with so called prognostically bad signs present (PBSP i.e. toxæmia, acidosis etc. as in Cases no. 1, 3 and 10) but were also found in those without (no. 9). In this small and rather uniform series the possible influence of the severity and the duration of the disease as judged by White's classification could not be assessed.

The results are somewhat at variance with those obtained by Sper<sup>+</sup> (1965). In a consecutive study of 13 diabetic pregnant women he found that the increase of forearm blood flow was of the same magnitude as in normal pregnancy but that the maximal values were reached already at 29–31 weeks of pregnancy whereafter a gradual decline took place. In the present series no such early peak was found. This discrepancy however may be because the severity of the disease in the individual cases may not be comparable in these small series. Furthermore Sper<sup>+</sup> used a plethysmographic water temperature of 36°C which tends to give a higher average forearm blood flow by vasodilatation in the skin as compared with 34°C used in the present study.

As in normal pregnancy the  $^{133}\text{Xe}$  clearance of forearm muscle in the diabetic women did not show any significant change during the course of pregnancy. The mean  $^{133}\text{Xe}$  muscle clearance at rest, 3.3 ml/100 g/min, was not significantly different from that of normal pregnant women, nor in fact from that of non-pregnant healthy women.  $^{133}\text{Xe}$  clearance can be assumed to be a measure of the effective capillary blood flow of skeletal muscle which accordingly does not seem to be altered during diabetic pregnancy. This is in principle in agreement with the results of Munck, Lindbjerg, Binder, Lassen and Trap-Jensen (1966) who studied muscle blood flow with  $^{133}\text{Xe}$  clearance technique in non-pregnant diabetics and found no difference in resting blood flow or maximal blood flow capacity as compared with non-diabetics.

As  $^{133}\text{Xe}$  is freely diffusible its clearance rate is limited only by the rate of capillary blood flow and not by the state of the capillary membrane. Trap-Jensen, Alpert, del Rio and Lassen (1967) found an increased capillary diffusion capacity for sodium ( $^{24}\text{Na}$ ) in diabetics, which they attributed to the capillary angopathy. No similar studies during diabetic pregnancy are available. Sperz (1965) however found a decreased capillary filtration capacity as measured by plethysmography in diabetic pregnancy suggesting a disturbed function of the capillary membrane.

In the second half of both normal and diabetic pregnancy the forearm blood flow as recorded with a plethysmograph increased gradually while the muscle blood flow as judged from  $^{133}\text{Xe}$  clearance remained constant. The interpretation of this finding is not as simple as it may seem. It may be explained by the assumption that in those pregnant diabetic cases where a reduced peripheral blood flow is observed, this reduction takes place essentially in the skin. As has been discussed earlier (Sperz and Jansson 1969) such a conclusion implies a distribution of peripheral blood flow in late pregnancy which cannot be accepted unreservedly. The question needs further elucidation.

The really important problem in diabetic pregnancy is how the disease affects the foeto-placental unit. The high foetal mortality in connection with advanced vascular disease in diabetes has led to the assumption that there is an inadequate uteroplacental blood flow (cf White 1949, 1965). In the present study the average myometrial capillary blood flow in diabetic pregnancy as measured by  $^{133}\text{Xe}$  clearance, 20.3 ml/100 g/min, was not significantly different from that of normal pregnancy 18.6 ml. The same is true for  $^{133}\text{Xe}$  clearance in the myometrium at the placental implantation site, which in diabetic pregnancy was found to be 24.3 ml, and for myometrium at a distance from placenta, the  $^{133}\text{Xe}$  clearance in which was 12.1 ml/100 g/min.

Considering the influence of the placenta on the myometrial circulation, it is unfortunate that the placental site could be determined only in a limited number of the cases investigated. This disadvantage however was to a certain extent eliminated by the fact that the  $^{133}\text{Xe}$  clearance measurements were repeated in the same area of the uterine wall, making the placental influ-

blood flow was significantly lower. As can be seen from Fig. 2, however, there was a wide scatter of the individual values some falling within the normal range. This is in agreement with the fact that only some of the patients in the age group studied may be expected to show vascular lesions severe enough to influence the rate of blood flow. The low values were often recorded in cases with so called prognostically bad signs present (PBSP, i.e. toxæmia, acidosis etc. as in Cases no. 1, 3 and 10) but were also found in those without (no. 9). In this small and rather uniform series the possible influence of the severity and the duration of the disease as judged by White's classification could not be assessed.

The results are somewhat at variance with those obtained by Spetz (1965). In a consecutive study of 13 diabetic pregnant women he found that the increase of forearm blood flow was of the same magnitude as in normal pregnancy but that the maximal values were reached already at 29–31 weeks of pregnancy whereafter a gradual decline took place. In the present series no such early peak was found. This discrepancy however may be because the severity of the disease in the individual cases may not be comparable in these small series. Furthermore Spetz used a plethysmographic water temperature of 36°C which tends to give a higher average forearm blood flow by vasodilatation in the skin as compared with 34°C used in the present study.

As in normal pregnancy the  $^{133}\text{Xe}$  clearance of forearm muscle in the diabetic women did not show any significant change during the course of pregnancy. The mean  $^{133}\text{Xe}$  muscle clearance at rest, 3.3 ml/100 g/min, was not significantly different from that of normal pregnant women nor in fact from that of non-pregnant healthy women.  $^{133}\text{Xe}$  clearance can be assumed to be a measure of the effective capillary blood flow of skeletal muscle which accordingly does not seem to be altered during diabetic pregnancy. This is in principle in agreement with the results of Munck, Lindbjerg, Binder, Lassen and Trap-Jensen (1966) who studied muscle blood flow with  $^{133}\text{Xe}$  clearance technique in non-pregnant diabetics and found no difference in resting blood flow or maximal blood flow capacity as compared with non-diabetics.

ever is also influenced by the state of the placental vessels. While some investigators have demonstrated the same vascular changes in diabetic placentas as in other tissues, others have not been able to confirm their findings (*cf* Warren *et al.* 1966). Berglund and Zetterström (1954) found a low oxygen content in umbilical venous blood in babies of diabetic mothers at Caesarean section, while Peol (1962) in studying oxygen content and saturation of cord blood and hemoglobin levels at birth found no evidence that placental insufficiency and foetal hypoxia was an important cause of intrauterine foetal death in diabetic pregnancy.

### SUMMARY

1 In 16 pregnancies complicated by diabetes mellitus repeated measurements of resting forearm blood flow by plethysmography and of forearm muscle blood flow by  $^{133}\text{Xe}$  clearance technique were performed between 13 and 37 weeks of gestation.

2 The myometrial circulation was evaluated in 18 pregnant diabetic women by consecutive measurements of  $^{133}\text{Xe}$  myometrial clearance between 20 and 39 weeks of pregnancy.

3 The total forearm blood flow at rest increased at the same rate in diabetic as in normal pregnancy. The average forearm blood flow however was significantly lower in the diabetic women. The capillary muscle blood flow measured by  $^{133}\text{Xe}$  clearance on the other hand, was of the same magnitude as in normal pregnancy and in healthy non-pregnant women and it remained constant during the course of pregnancy. The significance of these observations is discussed.

4 The myometrial blood flow as judged from  $^{133}\text{Xe}$  clearance was not significantly different from the values found in normal pregnancy either at the placental site or in non-placental myometrium. The capillary myometrial blood flow per unit weight of tissue was unchanged throughout pregnancy.

### Acknowledgements

This study was aided by grants from the Medical Faculty of the University of Göteborg, the Swedish Medical Research Council

ence relatively constant from one examination to another in the individual patient. From Fig 3 it can also be seen that the  $^{133}\text{Xe}$  clearance values in cases of undetermined placental site have a tendency to be distributed at two different levels, probably representing placental and non placental myometrium. Most values fall within the range of placental myometrium and no value below the range of non placental myometrium which makes it highly probable that the myometrial blood flow in diabetic pregnancy is at least not lower than in normal pregnancy. As in normal pregnancy no change in myometrial blood flow per unit weight of tissue during the course of pregnancy could be demonstrated.

The results are a confirmation of those of *Brudenell et al.* (1961) who in a study of 68 pregnant diabetic women with  $^{24}\text{Na}$  clearance were not able to detect any difference in myometrial circulation between these and a group of normal pregnant women. They did, however find a shorter  $^{24}\text{Na}$  clearance time in cases which subsequently resulted in foetal death than in those cases where the baby survived. The authors could not explain this unexpected finding. In view of the above mentioned observation of *Trap-Jensen et al.* (1967) the short  $^{24}\text{Na}$  clearance time may perhaps be explained by an increased sodium diffusion capacity indicating advanced capillary damage in the myometrium in association with foetal death.

Histopathological studies of the myometrium in diabetes seem to be very few. While *White* (1965) claims that the small myometrial blood vessels age very rapidly in diabetes *Pinkerton* (1963) found narrowing of the arterioles in the placental bed in only 3 out of 54 diabetic mothers. The normal myometrial circulation in the placental bed found in the present study agrees with the latter observation.

It must be remembered that  $^{133}\text{Xe}$  clearance gives a measure only of myometrial circulation. The major and most important part of uterine blood flow is directed via the spiral arteries to the chorio-decidual space. This fraction may be reduced in diabetic pregnancy in spite of a normal myometrial blood flow although it is reasonable to assume that parallel changes occur in the two vascular systems. The oxygen supply to the foetus how

## THE PROPHYLAXIS OF FOLATE DEFICIENCY IN PREGNANCY

BY

BRYAN M. HIBBARD AND ELIZABETH D. HIBBARD

Increasing awareness of the frequency of folate deficiency in pregnancy and of its relationship with pregnancy complications such as abortion and abruptio placentae (Hibbard 1964) as well as with megaloblastic anaemia, has led to the extensive administration of folic acid supplements to pregnant women.

Until recently the commonly used dosage has been 5 mg given 1-3 times daily. Such dosage is almost certainly unnecessarily large. It may be dangerous if administered to patients with unrecognised Vitamin B<sub>12</sub> deficiency although this very slight risk has probably been over-emphasized in the past.

Recent trials have been designed to determine suitable prophylactic dosage in the microgramme range. Thus Chanarin and his colleagues (1965) in London found that a supplement of 20 µg/day was insufficient to prevent megaloblastic anaemia but subsequently found that in the majority of women 100 µg was an adequate supplement, as judged by reticulocyte count and serum folate levels (Chanarin et al., 1968). Hansen and Rybo (1967) also found that a supplement of 100 µg was adequate. Willoughby and Jewell (1966) investigating a population of poorer social and nutritional status in Glasgow suggested that supplementation in the order of 300 µg folic acid daily is desirable. Chisholm (1966) found no significant difference in folate status between patients given supplements of 5 mg and 500 µg.

Two particular problems arise in the conduct of such trials. The first, commented on by Chisholm, is the difficulty in assessing the value of prophylactic therapy in a population with little evidence

and the Medical Society of Göteborg The statistical analysis was performed by Mrs Gull Britt Palm fil. kand.

## REFERENCES

- Bårdny F R. *Acta Med. Scand., Suppl.* 304 1955  
 Bishop E H. *Amer J Obstet. Gynec.* 96 863 1966  
 Berglund, G. and Zetterström R. *Acta Paediat.* 43 368 1954  
 Brownlee A. A. *Statistical Theory and Methodology in Science and Engineering*, New York, 1965  
 Brudenell J M, Miles J M. and Coleman A. *J Obstet. Gynec. Brit. Cwth* 68 238, 1961  
 Falk V, Forkman B. and Lindell S E. *Strahlentherapie (Sonderb.)* 65 162, 1967  
 Guilhem P, Pontonnier A., and Pontonnier G. *Gynec. Obstét. (Paris)* 64 313 1965  
 Jansson I. *Acta Obstet. Gynec. Scand.* 48 302 1969  
 Lassen N A., Lindbjerg, I. and Munk O. *Lancet* 1 686 1964  
 Lysgaard H. and Lefèvre H. *Acta Obstet. Gynec. Scand.* 44 401 1965  
 Megibow R. S. Megibow S J, Pollack H, Bookman, J J. and Osberman K. *Amer J Med.* 15 322, 1953  
 Mendlowitz M, Grossman E. B. and Alpert S. *Amer J Med.* 15 316 1953  
 Munk O, Lindbjerg, I, Binder C, Lassen N A. and Trap-Jensen, J. *Diabetes* 15 323 1966  
 Pedersen J. *The Pregnant Diabetic and Her Newborn*, Munksgaard, Copenhagen 1967  
 Pedersen J. and Pedersen L. M. *Acta Endocrin.* 50 70 1965  
 Fecl J. *Amer J Obstet. Gynec.* 83 847 1962  
 Pinkerton J H M. *Proc. Roy Soc. Med.* 56 1021 1963  
 Sigroth A. *Acta Med. Scand. Suppl.* 325 1957  
 Spetz S. *Acta Obstet. Gynec. Scand.* 43 309 1964  
 Spetz S. *Acta Obstet. Gynec. Scand.* 44 suppl. 1 1965  
 Spetz S. and Jansson I. *Acta Obstet. Gynec. Scand.* 48 285 1969  
 Trap-Jensen J, Alpert J S, del Rio G. and Lassen, N A. *Acta Med. Scand., Suppl.* 476 135 1967  
 Warren S, LeCompte P M. and Legg, M A. *The Pathology of Diabetes Mellitus* Lea & Febiger Philadelphia 1966  
 Weber H W. and Wicht C L. *S. Afric J Lab. Clin. Med.* 8 83 1967  
 White P. *Amer J Med.* 7 609 1949  
 White P. *Diabetes* 7 494 1958  
 White P. *Med Clin. N Amer* 49 1015 1965

Received on Jan. 15 1969



## THE PROPHYLAXIS OF FOLATE DEFICIENCY IN PREGNANCY

BY

BRYAN M. HIBBARD AND ELIZABETH D. HIBBARD

Increasing awareness of the frequency of folate deficiency in pregnancy and of its relationship with pregnancy complications such as abortion and abruptio placentae (Hibbard 1964) as well as with megaloblastic anaemia, has led to the extensive administration of folic acid supplements to pregnant women.

Until recently the commonly used dosage has been 5 mg given 1-3 times daily. Such dosage is almost certainly unnecessarily large. It may be dangerous if administered to patients with unrecognized Vitamin B<sub>12</sub> deficiency although this very slight risk has probably been over-emphasized in the past.

Recent trials have been designed to determine suitable prophylactic dosage in the microgramme range. Thus Chanarin and his colleagues (1965) in London found that a supplement of 20 µg/day was insufficient to prevent megaloblastic anaemia, but subsequently found that in the majority of women 100 µg was an adequate supplement, as judged by reticulocyte count and serum folate levels (Chanarin et al., 1968). Hansen and Rybo (1967) also found that a supplement of 100 µg was adequate. Willoughby and Jewell (1966) investigating a population of poorer social and nutritional status in Glasgow suggested that supplementation in the order of 300 µg folic acid daily is desirable. Chisholm (1966) found no significant difference in folate status between patients given supplements of 5 mg and 500 µg.

Two particular problems arise in the conduct of such trials. The first commented on by Chisholm, is the difficulty in assessing the value of prophylactic therapy in a population with little evidence

of folate deficiency the incidence of megaloblastic anaemia of pregnancy in her population being less than 1 per cent. For this reason very large numbers of patients may be required to obtain significant results.

The second difficulty arises from the fact that the daily dietary intake of folates is uncertain but may range from less than 50  $\mu\text{g}$  to several hundred microgrammes. Thus the nature of the diet may influence considerably the minimum supplementary requirements. This probably accounts for the variation in results of trials in different areas.

The aim of prophylactic therapy should be to eliminate the occurrence of deficiency and the optimal dosage should be the minimum required to maintain a satisfactory folate balance in patients at known increased risk namely those with an insignificant dietary intake and with other factors such as high parity and previous anaemia especially if known to have been associated with folate depletion. Allowance must also be made for the fact that in clinical practice as opposed to personally supervised trials, tablets are rarely taken with the recommended frequency.

From available evidence it was concluded that a daily supplement of 500  $\mu\text{g}$  folic acid should be satisfactory for the prevention of folate deficiency even in high risk patients and a triple blind trial was instituted to test this hypothesis.

### *Clinical material*

Mill Road Maternity Hospital Liverpool serves a population of predominantly poor social status, low nutritional standards and high parity. In a previous investigation the incidence of megaloblastic anaemia was shown to be approximately 5 per cent and biochemical evidence of defective folate metabolism, as judged by FIGLU excretion, was demonstrated in 10–15 per cent of the hospital population (Hibbard 1964). Recurrence of defective metabolism was found in 73 per cent subsequent pregnancies (Hibbard and Hibbard 1966).

Only patients with a known history of defective folate metabolism in a previous pregnancy as judged by megaloblastic anaemia

excessive FIGLU excretion or low serum folate level ( $< 2$  ng/ml) were admitted to the trial. Selection of high risk patients in this way eliminates many of the problems found by other investigators in obtaining significant results.

No patient was admitted to the trial after 20 weeks gestation or if the haemoglobin level was 10.5 g per 100 ml or less. Patients were divided into three groups and received gelatine-coated capsules of identical appearance containing either 500  $\mu$ g folic acid (Pregfol Wyeth) 5.0 mg folic acid or a placebo. In addition all capsules contained 60 mg elemental iron as ferrous sulphate. Dosage was one capsule daily. The patients were allocated consecutive numbers and the three capsules were issued by blind random selection at the source of issue. The code was not known whilst the trial was in progress. In view of the high risk of these women developing folate depletion, careful and detailed supervision by one or two clinicians was maintained as far as possible.

### *Laboratory Investigations*

Response to treatment was judged by assay of urinary formiminoglutamic acid (FIGLU) excretion and by serum folate levels at 4 week intervals. *Urinary FIGLU Excretion* was assessed following a 10 g histidine load. The method and assay using high voltage electrophoresis were as detailed by Hibbard (1964). Excretion of more than 20 g/ml FIGLU in the urine passed 5 hours after histidine load was reported as a positive test. *Serum Folate Level* was measured on venous blood collected into folate-free sterile glass containers. Serum was stored at  $-20^{\circ}\text{C}$  without the addition of ascorbic acid. Assay was performed as soon as possible after collection of the specimen using a modification of Herbert's (1961) aseptic addition method. Using this technique we have found the normal range of values in non-pregnant subjects is 7-20 ng/ml. Values in pregnant patients fall to lower levels but for purposes of this investigation serum folate levels of less than 40 ng/ml were regarded as abnormal.

In addition haemoglobin estimation was performed on capillary blood using an EEL haemoglobinometer and an oxy-haemoglobin

Table 1. *Results of Daily Prophylactic Administration to High Risk Patients of 60 mg Elemental Iron Alone and With 500 µg and 5 mg Folic Acid*

	No Folic Acid	500 µg Folic Acid	5 mg Folic Acid
Initial admissions to trial	26	27	26
Removed from trial after first attendance	8	10	5
Active participants with serial observations	18	17	21
Folate deficiency (FIGLU positive ± serum folate < 4 ng/ml) whilst on therapy	7	0	0
Secondary defaulters — non attendance or failure to take therapy	4	7	8
Folate deficiency subsequently shown in secondary defaulters	4	2	5

method. Values less than 10.5 g per 100 ml were regarded as indicative of anaemia.

Haemoglobin estimations were performed on every patient at each antenatal visit, this being the usual practice in the hospital.

### *Results*

Seventy-nine patients were admitted to the trial but of these 23 were subsequently excluded the principle reasons being default (12) and a positive FIGLU excretion test at the first visit (7). A further 14 patients defaulted subsequently but the results of treatment for at least 4 weeks were known in these patients.

The serial results of serum folate assays and FIGLU excretion tests are shown in Figures 1-3.

### *Control patients*

In the control group of patients not receiving folic acid it is observed that the serum folate levels tend to fall as pregnancy progresses (Fig. 1) a trend noted by other investigators and thus in spite of the fact that patients who developed excessive FIGLU

SERUM FOLATE LEVELS  
PROPHYLACTIC TRIAL NO FOLIC ACID SUPPLEMENT

FIGURE 1 at p. 1

M. et al.

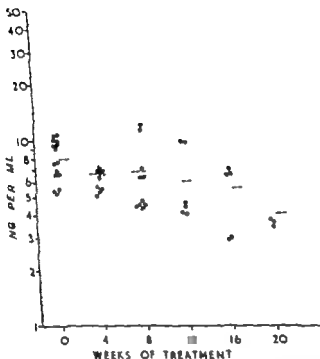


Fig. 1 Serial serum folate levels in patients with high risk of folate deficiency treated with 60 mg elemental iron daily. Patients developing folate deficiency were subsequently withdrawn from the trial and treated with folic acid.

excretion were removed from the trial. The mean serum folate level at the time of admission to the trial was 8.0 ng/ml, but fell to 6.0 ng/ml 12 weeks later and after 20 weeks had fallen to 4.0 ng/ml.

Of the 26 patients initially admitted to the control group 5 were lost by primary default and 1 was given folic acid by her family doctor. Of the remaining 20 patients, 2 had positive

Table I. *Results of Daily Prophylactic Administration to High Risk Patients of 60 mg Elemental Iron Alone and With 500 µg and 5 mg Folic Acid*

	No Folic Acid	500 µg Folic Acid	5 mg Folic Acid
Initial admissions to trial	26	27	26
Removed from trial after first attendance	8	10	5
Active participants with serial observations	18	17	21
Folate deficiency (FIGLU positive $\pm$ serum folate < 4 ng/ml) whilst on therapy	7	0	0
Secondary defaulters — non attendance or failure to take therapy	4	2	8
Folate deficiency subsequently shown in secondary defaulters	4	2	5

method. Values less than 10.5 g per 100 ml were regarded as indicative of anaemia.

Haemoglobin estimations were performed on every patient at each antenatal visit, this being the usual practice in the hospital.

### Results

Seventy-nine patients were admitted to the trial but of these 23 were subsequently excluded, the principle reasons being default (12) and a positive FIGLU excretion test at the first visit (7). A further 14 patients defaulted subsequently but the results of treatment for at least 4 weeks were known in these patients.

The serial results of serum folate assays and FIGLU excretion tests are shown in Figures 1–3.

### Control patients

In the control group of patients not receiving folic acid it is observed that the serum folate levels tend to fall as pregnancy progresses (Fig. 1), a trend noted by other investigators and this in spite of the fact that patients who developed excessive FIGLU

SERUM FOLATE LEVELS  
PROPHYLACTIC TRIAL NO FOLIC ACID SUPPLEMENT  
 FIGURE 1 post 11  
 H I

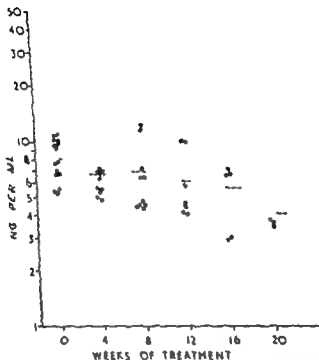


Fig 1 Serial serum folate levels in patients with high risk of folate deficiency treated with 60 mg elemental iron daily. Patients developing folate deficiency were subsequently withdrawn from the trial and treated with folic acid.

excretion were removed from the trial. The mean serum folate level at the time of admission to the trial was 8.0 ng/ml, but fell to 6.0 ng/ml 12 weeks later and after 20 weeks had fallen to 4.0 ng/ml.

Of the 26 patients initially admitted to the control group 5 were lost by primary default, and 1 was given folic acid by her family doctor. Of the remaining 20 patients, 2 had positive

Table 1. *Results of Daily Prophylactic Administration to High Risk Patients of 60 mg Elemental Iron Alone and With 500 µg and 5 mg Folic Acid*

	No Folic Acid	500 µg Folic Acid	5 mg Folic Acid
Initial admissions to trial	26	27	26
Removed from trial after first attendance	8	10	5
Active participants with serial observations	18	17	21
Folate deficiency (FIGLU positive ± serum folate < 4 ng/ml) whilst on therapy	7	0	0
Secondary defaulters — non attendance or failure to take therapy	4	2	8
Folate deficiency subsequently shown in secondary defaulters	4	2	5

method. Values less than 10.5 g per 100 ml were regarded as indicative of anaemia.

Haemoglobin estimations were performed on every patient at each antenatal visit this being the usual practice in the hospital.

### Results

Seventy nine patients were admitted to the trial but of these 23 were subsequently excluded the principle reasons being default (12) and a positive FIGLU excretion test at the first visit (7). A further 14 patients defaulted subsequently but the results of treatment for at least 4 weeks were known in these patients.

The serial results of serum folate assays and FIGLU excretion tests are shown in Figures 1-3

### Control patients

In the control group of patients not receiving folic acid it is observed that the serum folate levels tend to fall as pregnancy progresses (Fig 1) a trend noted by other investigators, and this in spite of the fact that patients who developed excessive FIGLU



SERUM FOLATE LEVELS  
PROPHYLACTIC TRIAL NO FOLIC ACID SUPPLEMENT

FIGURE 1

— Me — les

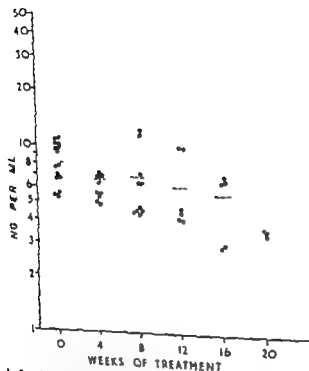


Fig 1 Serial serum folate levels in patients with high risk of folate deficiency treated with 60 mg elemental iron daily. Patients developing folate deficiency were subsequently withdrawn from the trial and treated with folic acid.

excretion were removed from the trial. The mean serum folate level at the time of admission to the trial was 8.0 ng/ml, but fell to 6.0 ng/ml 12 weeks later and after 20 weeks had fallen to 4.0 ng/ml.

Of the 26 patients initially admitted to the control group 5 were lost by primary default, and 1 was given folic acid by her family doctor. Of the remaining 20 patients 2 had positive

Table 1. *Results of Daily Prophylactic Administration to High Risk Patients of 60 mg Elemental Iron Alone and With 500 µg and 5 mg Folic Acid*

	No Folic Acid	500 µg Folic Acid	5 mg Folic Acid
Initial admissions to trial	26	27	26
Removed from trial after first attendance	6	10	5
Active participants with serial observations	18	17	21
Folate deficiency (FIGLU positive ± serum folate < 4 ng/ml) whilst on therapy	7	0	0
Secondary defaulters — non attendance or failure to take therapy	4	2	8
Folate deficiency subsequently shown in secondary defaulters	4	2	5

method. Values less than 10.5 g per 100 ml were regarded as indicative of anaemia.

Haemoglobin estimations were performed on every patient at each antenatal visit this being the usual practice in the hospital.

### *Results*

Seventy nine patients were admitted to the trial but of these 23 were subsequently excluded the principle reasons being default (12) and a positive FIGLU excretion test at the first visit (7). A further 14 patients defaulted subsequently but the results of treatment for at least 4 weeks were known in these patients.

The serial results of serum folate assays and FIGLU excretion tests are shown in Figures 1-3.

### *Control patients*

In the control group of patients not receiving folic acid it is observed that the serum folate levels tend to fall as pregnancy progresses (Fig. 1) a trend noted by other investigators and this in spite of the fact that patients who developed excessive FIGLU

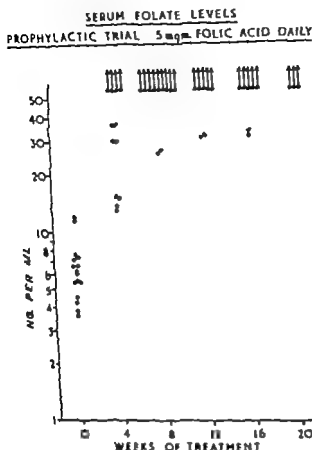


Fig. 3 Serial serum folate levels in patients with a high risk of folate deficiency treated with 5 mg folic acid and 60 mg elemental iron daily

#### *Patients receiving folic acid*

In both groups of patients receiving folic acid, serum folate levels rose within 4 weeks and generally were maintained at high levels thereafter (Figs. 2 and 3). Mean values could not be calculated, as many readings were above levels amenable to accurate assay. But median values, after the initial rise, showed no significant subsequent variations, even with prolonged therapy.

In the 500  $\mu$ g dosage group the levels generally were above the

SERUM FOLATE LEVELS  
PROPHYLACTIC TRIAL - 500  $\mu$ g FOLIC ACID DAILY

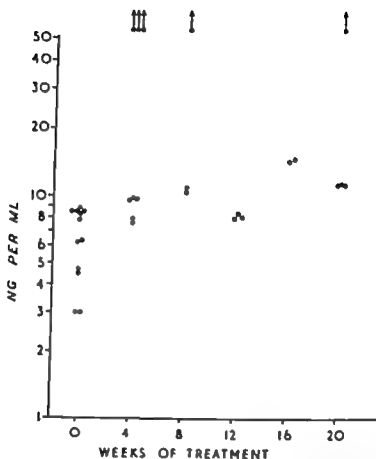


Fig. 1. Serial serum folate levels in patients with a high risk of folate deficiency treated with 500  $\mu$ g folic acid and 60 mg elemental iron daily.

FIGLU excretion tests at their first visit and eleven developed positive tests subsequently. Thus positive FIGLU excretion tests were found ultimately in 13 out of 20 patients available for serial study. This incidence of 65 per cent is close to the expected incidence of 73 per cent found in a previous study of recurrent defective folate metabolism in the same population (Hibbard and Hibbard 1966). Eleven of the 18 active participants in the control series showed excessive FIGLU excretion as the trial progressed.

excretion tests associated in 2 instances with serum folate levels below 4 ng/ml. Seven patients under active treatment with folic acid were anaemic but all had high serum folate levels and negative FIGLU excretion tests.

Apart from the patients with positive FIGLU tests, treatment was continued as before with the addition of a further 120 mg elemental iron daily as ferrous sulphate. In those cases available for adequate follow up thereafter a significant rise in haemoglobin level was shown.

### Discussion

This study of groups of pregnant women, selected because of a high risk of defective folate metabolism, shows that 500 g folic acid daily is adequate to prevent folate deficiency during pregnancy and is as effective as a dosage of 5 mg daily as judged by the incidence of excessive FIGLU excretion the serum folate levels and the haemoglobin concentration. The exceptionally high serum folate levels found in many patients receiving 5 mg folic acid daily do not appear to confer any clinical benefit.

It is evident from the figures in Table 1 that the population studied, as well as being nutritionally and economically inferior is characterized by a lax attitude towards personal health. In spite of close personal supervision it is notable that of the initial 79 patients 12 failed to attend the clinic again after the first visit and subsequently a further 14 defaulted or were known to have failed to take the prescribed therapy.

It is therefore reasonable to assume that amongst the remaining patients there were many who did not take their capsules with the prescribed frequency. Yet no case of folate depletion occurred amongst those patients receiving 500 g folic acid daily suggesting that this dosage provides a reasonable safety margin even for the high risk poorly motivated patient.

Whilst the formulation suggested provides adequate protection from folate deficiency it may be questioned whether the iron content is adequate for a poorly nourished population, since some patients developed mild anaemia, presumably of iron deficient type. Since the majority of these patients responded to treatment

Table II. Serum Folate Levels Approximately 4 Weeks After Commencing Prophylactic Therapy

	No Folic Acid	500 $\mu$ g Folic Acid	5 mg Folic Acid
Patients investigated	18	17	21
Serum folate over 50 ng/ml	0	3	7
Serum folate over 10 ng/ml	2	7	20
Serum folate 4-10 ng/ml	14	8	1
Serum folate under 4 ng/ml	2	2	0

normal range but rarely reached the exceptionally high values found in the 5 mg dosage group. Two patients receiving 500  $\mu$ g daily had serum folate levels below 4 ng/ml 4 weeks or more after commencing therapy. One of these results was probably erroneous as subsequently assay showed a level of 8.9 ng/ml. The other patient showed repeatedly low levels with a maximum of 3.8 ng/ml although she denied failing to take the prescribed therapy.

None of the patients who were given folic acid supplements had positive FIGLU excretion tests whilst taking therapy regularly. However among the secondary defaulters both patients in the 500  $\mu$ g group and 5 of the 8 in the 5 mg group were shown to be folate deficient when further opportunities for investigation occurred later in pregnancy.

The mean haemoglobin levels in all groups showed consistent but small decreases from the initial readings.

Twenty weeks after commencing therapy the mean decrease in haemoglobin level was as follows:

No folic acid	0.75 G/100 ml
500 $\mu$ g daily	1.5 G/100 ml
5 mg daily	1.6 G/100 ml

These differences are not statistically significant.

There was no case of severe anaemia, the lowest haemoglobin level recorded being 9.6 G/100 ml. However a haemoglobin concentration consistently below 10.5 G/100 ml was found in 7 patients in the control group and 4 of these had positive FIGLU

excretion tests associated in 2 instances with serum folate levels below 4 ng/ml. Seven patients under active treatment with folic acid were anaemic but all had high serum folate levels and negative FIGLU excretion tests.

Apart from the patients with positive FIGLU tests treatment was continued as before with the addition of a further 120 mg elemental iron daily as ferrous sulphate. In those cases available for adequate follow up thereafter a significant rise in haemoglobin level was shown.

### Discussion

This study of groups of pregnant women selected because of a high risk of defective folate metabolism, shows that 500  $\mu$ g folic acid daily is adequate to prevent folate deficiency during pregnancy and is as effective as a dosage of 5 mg daily as judged by the incidence of excessive FIGLU excretion the serum folate levels and the haemoglobin concentration. The exceptionally high serum folate levels found in many patients receiving 5 mg folic acid daily do not appear to confer any clinical benefit.

It is evident from the figures in Table I that the population studied, as well as being nutritionally and economically inferior is characterized by a lax attitude towards personal health. In spite of close personal supervision it is notable that of the initial 79 patients 12 failed to attend the clinic again after the first visit and subsequently a further 14 defaulted or were known to have failed to take the prescribed therapy.

It is therefore reasonable to assume that amongst the remaining patients there were many who did not take their capsules with the prescribed frequency. Yet no case of folate depletion occurred amongst those patients receiving 500  $\mu$ g folic acid daily suggesting that this dosage provides a reasonable safety margin even for the high risk poorly motivated patient.

Whilst the formulation suggested provides adequate protection from folate deficiency it may be questioned whether the iron content is adequate for a poorly nourished population since some patients developed mild anaemia presumably of iron deficient type. Since the majority of these patients responded to treatment

with additional iron it is concluded that more satisfactory prophylaxis of anaemia would be achieved by a formulation offering a higher dosage of elemental iron providing that this does not result in an increase in adverse gastro-intestinal symptoms.

### SUMMARY

Even in bad risk populations a regime offering a single daily capsule comprising 500  $\mu$ g folic acid and 60 mg elemental iron gives effective protection from folate deficiency but may not be invariably effective in preventing iron deficiency. However if satisfactory prophylaxis of anaemia as well as folate deficiency is to be achieved a higher iron content may be considered desirable.

### Acknowledgements

We are indebted to the Medical Research Council and the Research Committee of the United Liverpool Hospitals for grants supporting the laboratory investigations reported here.

We are also grateful to Dr D. Richards and Messrs John Wyeth and Co. Ltd for the preparation and supply of the haematinic capsules.

### REFERENCES

- Chasnarin L, Rothman D and Berry V. *Brit. Med. J.* 1: 490, 1965.  
Chasnarin L, Rothman D, Wed A. and Perry J. *Brit. Med. J.* 1: 390, 1968.  
Christholm M. *J. Obstet. Gynaec. Brit. Commonw.* 73: 191, 1966.  
Hansen H. and Rybo G. *Acta obst. et gynec. scandinav.* 46: Suppl. 7: 107, 1967.  
Herbert V. *J. Clin. Invest.* 40: 81, 1961.  
Hibbard B. M. *J. Obstet. Gynaec. Brit. Commonw.* 71: 529, 1964.  
Hibbard B. M. and Hibbard E. D. *J. Obstet. Gynaec. Brit. Commonw.* 73: 428, 1966.  
Hibbard E. D. *Lancet* ii: 1146, 1964.  
Willoughby M. L. N. and Jewell F. J. *Brit. Med. J.* ii: 1568, 1966.



## THE TREATMENT OF FOLATE DEFICIENCY IN PREGNANCY

BY

BRYAN M. HIBBARD AND ELIZABETH D. HIBBARD

The efficacy of daily supplements of folic acid in the microgramme range for the prophylaxis of folate deficiency in pregnancy is now well accepted but lower dosage regimes have not been so widely adopted for the treatment of established folate deficiency.

The purpose of the trial described here was to assess the value of oral treatment of folate deficiency with 500 µg folic acid three times daily as compared with the traditional dosage of 5 mg three times daily.

### Clinical material

All the patients admitted to the trial were booked for confinement at Mill Road Maternity Hospital, Liverpool and their gestation period was less than 36 weeks. The primary criterion for admission was the demonstration of excessive urinary FIGLU excretion, assayed by high voltage electrophoresis (Hibbard 1964). This was in most cases associated with anaemia and/or low serum folate levels or an obstetric history suggestive of defective folate metabolism. The investigation was conducted as a double blind trial and patients were allotted to treatment groups randomly. Two types of capsule of identical appearance were used. Both contained 60 mg elemental iron as ferrous sulphate but the folic acid content was either 500 µg or 5 mg. The capsules were prescribed to be taken three times daily so that patients all received 180 mg elemental iron and either 1.5 mg or 15 mg folic acid daily.

Table 1 *Results of Treatment of Defective Folate Metabolism with 180 mg Elemental Iron Plus 1.5 mg or 15 mg Folic Acid Daily*

	1.5 mg Folic Acid + 180 mg Iron Daily	15 mg Folic Acid + 180 mg Iron Daily
Initial admissions to trial	97	100
Subsequently excluded from trial	33	26
Active participants		
with serial observations	64	74
FIGLU test positive after 4 weeks therapy or became positive again later in pregnancy	5 (8%)	6 (8%)
Low folate level initially (< 4 ng/ml)	33	43
Low folate after 4 weeks therapy	0/27 investigated	1/26 investigated
Low haemoglobin level initially (10.5 G/100 ml)	28	40
Low Hb after 4 weeks therapy	8	19
Low Hb after 8 weeks therapy	3/11 investigated	5/23 investigated

### *Laboratory investigations*

Serum folate levels and urinary FIGLU excretion were determined at 4 weekly intervals as detailed previously (Hibbard and Hibbard 1969). Haemoglobin concentration was estimated at every antenatal visit.

### *Results*

One hundred and ninety seven patients were admitted to the trial but of these 59 were subsequently excluded principally because of default (32) or because folic acid had been prescribed from other sources (19).

### *FIGLU excretion*

The FIGLU excretion test remained positive or again become positive later in pregnancy in 5 (8 per cent) of the low dosage group and 6 (8 per cent) of the high dosage group. In the former group all the serum folate levels were normal but 3 patients had

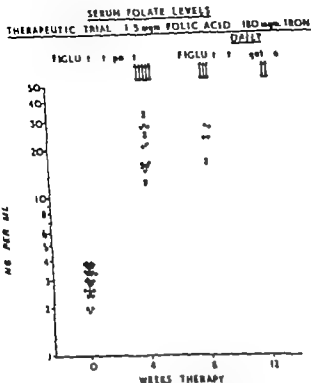


Fig. 1 Serial serum folate levels in patients with defective folate metabolism (initial serum folate level less than 4 ng/ml and excessive FIGLU excretion) treated with 1.5 mg folic acid and 180 mg elemental iron daily

haemoglobin concentrations under 10.5 G/100 ml. In the latter group 1 patient had a low serum folate (1.9 ng/ml) and 2 were anaemic.

#### *Serum folate levels*

The serum folate levels in both groups generally rose within 4 weeks and were maintained at normal or high levels. Because of limitations imposed by the assay method accurate readings above 50 ng/ml could not be obtained and therefore mean values could not be determined, but it is evident that after 4 weeks therapy



## HAEMOGLOBIN LEVELS IN PATIENTS WITH INITIAL HAEMOGLOBIN

CONCENTRATION &lt; 10.5 G/100 ML

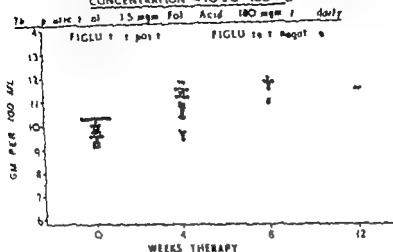


Fig. 3 Serial haemoglobin estimations in patients with initial haemoglobin concentrations under 10.5 G/100 ml treated with 1.5 mg folic acid and 180 mg elemental iron daily

(late level (Table 1). The solitary failure (folate 1.9 ng/ml) also had a positive FIGLU test and was anaemic. It seemed likely that she had not taken the prescribed therapy as a further folate assay gave a result of 2.3 ng/ml.

### Haemoglobin concentration

After 4 weeks of treatment both groups showed a statistically significant (at the 1 per cent level) rise in haemoglobin concentration. There was no significant difference between the responses obtained with low dosage and high dosage of folic acid, but there seemed to be greater variability in the results with the lower dosage.

The response of those patients with a low initial haemoglobin concentration (under 10.5 G/100 ml) was considered separately. In the low dosage group there were 28 such patients and 15 of these had a serum folate level below 4 ng/ml. After 4 weeks of

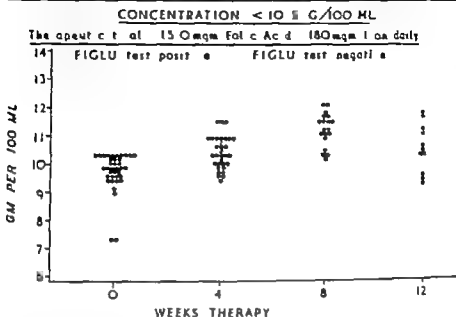
HAEMOGLOBIN LEVELS IN PATIENTS WITH INITIAL HAEMOGLOBIN

Fig. 4 Serial haemoglobin estimations in patients with initial haemoglobin concentrations under 10.5 G/100 ml treated with 15 mg folic acid and 180 mg elemental iron daily

therapy 8 of the 28 patients still had low haemoglobin levels although their folate levels and FIGLU excretion were normal. In the high dosage group 40 patients had an initial haemoglobin level below 10.5 G/100 ml and 24 of these had a serum folate level below 4 ng/ml. After 4 weeks therapy nineteen patients remained anaemic although only one of these had a positive FIGLU excretion test associated with a serum folate level of 1.9 ng/ml.

### *Discussion*

The most important observation in this comparative trial is that there was no significant difference in the efficacy of the low dosage and high dosage regimes as assessed by any of the parameters studied that is reversion of FIGLU excretion to normal, rise in serum folate level to above 4 ng/ml and correction of anaemia.

As judged biochemically the response was generally satisfac

tory but the persistence or recurrence of excessive FIGLU excretion in 8 per cent patients in both groups suggests that some patients have an intrinsic metabolic defect in addition to, or rather than, a deficient intake. This is supported by the fact that with one exception this group of patients had serum folate levels within or above the normal range at the time of their subsequent positive FIGLU excretion tests. Also 3 out of 5 patients in the low dosage group and 2 out of 6 in the high dosage had continued clinical evidence of anaemia. It is notable that this figure of 8 per cent failure to respond to high dosage oral folic acid therapy is exactly the same as that reported in a previous study in a large series of patients from the same hospital (Hibbard 1967). This emphasizes the need for continued clinical and laboratory supervision of patients for whom folic acid therapy has been prescribed.

The importance of factors other than dietary inadequacy of iron and folic acid, and the need for regular supervision is also shown by the poor haemoglobin response of some patients. The reasons for this are often easily determined, for example the presence of helminthic infestations, chronic insidious blood loss, or chronic urinary infection but sometimes less obvious factors such as inborn metabolic errors associated with defective haemoglobin synthesis operate.

Even the low dosage regime used in this trial may be unnecessarily large for effective therapy. The choice of dosage was based principally on theoretical considerations and the results of trials with microgramme dosage for prophylaxis. If 500  $\mu$ g daily was adequate for prophylaxis it was considered that three times this amount would provide sufficient folic acid for normal daily requirements, for the additional demands which would accompany the restoration of normal cellular activity and increased haemopoiesis and would enable depleted reserves to be replenished.

In addition, it is convenient to use the same formulation of tablet as is used for prophylaxis. The increased daily dose provides 150 mg elemental iron which should be adequate for the treatment of concomitant iron deficiency which is commonly seen in association with folate deficiency.

Further trials using even lower doses of folic acid would be of

interest to determine the theoretical needs for folic acid in depleted patients, but for widespread clinical application it must be born in mind that a reasonable safety margin is required and allowance must be made for variations in individual requirements, absorption and failure to take prescribed treatment. In the present study there was a suggestion of greater fluctuations in haemoglobin response amongst patients in the low dosage group and this could imply that the dosage was close to the requirements in some cases.

### SUMMARY

A trial comparing the value of daily doses of 1.5 mg and 15 mg folic acid given orally for the treatment of folate deficiency in pregnancy shows no significant differences between the two groups as judged by excessive FIGLU excretion, low serum folate levels and haemoglobin concentrations.

It is concluded that 1.5 mg folic acid daily is adequate for the treatment of folate deficiency in pregnancy. The importance of continued clinical and laboratory supervision of patients receiving treatment is emphasised since a small proportion of patients fail to show a satisfactory response to therapy even taking 15 mg folic acid daily.

### *Acknowledgements*

We are indebted to the Medical Research Council and the Research Committee of the United Liverpool Hospitals for grants supporting the laboratory investigations reported here.

We are also grateful to Dr D. Richards and Messrs. John Wyeth and Co. Ltd. for the preparation and supply of the haematinic capsules.

### REFERENCES

- Hibbard B. M. *Acta obst. et gynec. scandinav.* 46 Suppl. 7: 47, 1967.  
Hibbard B. M. and Hibbard E. D. 48: 339, 1969.  
Hibbard E. D. *Lancet* i: 1146, 1964.



## GASTROINTESTINAL PROTEIN LOSS IN TOXAEMIC PATIENTS

BY

L. LAAKSO AND E. PAASIO

Semi-quantitative radioactive isotope studies have given significant additional diagnostic help in the analysis of intestinal plasma protein loss. The most common techniques involve the use of iodine-131 labelled albumin (RIHSA 131) (Jarnum 1961a) or Polyvinylpyrrolidone (PVP 131) (Gordon 1959) and the measurement of radioactive 131 in the faeces. Intestinal plasma protein loss is a non-specific finding associated with several diseases of the gastrointestinal tract. For example massive protein loss in the faeces may be caused by hypertrophic gastritis, gastric carcinoma, the sprue syndrome, ulcerative colitis, salmonellosis, lymphomas, obstruction of the thoracic duct, essential intestinal lymphangectasia and a fall in venous pressure in the systemic circulation (Schwarz et al. 1967). Plasma protein loss is also caused by certain general conditions accompanied by disturbances in capillary permeability such as shock, radiation injuries and nephrosis (Baranum et al. 1965). In view of these facts we decided to study the possible plasma protein loss in the faeces of toxæmic patients.

### *Material and Method*

Sixteen parturients were studied. Six had normal pregnancies, four had preeclampsia of pregnancy and six had preeclampsia with toxæmia. A total of 44 faecal determinations were made. Each subject was given immediately post partum 15 drops of potas-

interest to determine the theoretical needs for folic acid in depleted patients but for widespread clinical application it must be born in mind that a reasonable safety margin is required and allowance must be made for variations in individual requirements, absorption and failure to take prescribed treatment. In the present study there was a suggestion of greater fluctuations in haemoglobin response amongst patients in the low dosage group and this could imply that the dosage was close to the requirements in some cases.

### SUMMARY

A trial comparing the value of daily doses of 1.5 mg and 15 mg folic acid given orally for the treatment of folate deficiency in pregnancy shows no significant differences between the two groups as judged by excessive FIGLU excretion low serum folate levels and haemoglobin concentrations.

It is concluded that 1.5 mg folic acid daily is adequate for the treatment of folate deficiency in pregnancy. The importance of continued clinical and laboratory supervision of patients receiving treatment is emphasised since a small proportion of patients fail to show a satisfactory response to therapy even taking 15 mg folic acid daily.

### Acknowledgements

We are indebted to the Medical Research Council and the Research Committee of the United Liverpool Hospitals for grants supporting the laboratory investigations reported here.

We are also grateful to Dr D Richards and Messrs. John Wyeth and Co Ltd. for the preparation and supply of the haematinic capsules.

### REFERENCES

- Hibbard B M. *Acta obst et gynec scandinav* 46 Suppl 7 47 1967
- Hibbard, B. M. and Hibbard E. D. 48 339 1969
- Hibbard E. D. *Lancet* i 1146 1964

## GASTROINTESTINAL PROTEIN LOSS IN TOXAEMIC PATIENTS

BY

L. LAAKSO AND I. PAASIO

Semi-quantitative radioactive isotope studies have given significant additional diagnostic help in the analysis of intestinal plasma protein loss. The most common techniques involve the use of Iodine 131 labelled albumin (RIHSA 131) (Jarnum 1961 a) or Polyvinylpyrrolidone (PVP 131) (Gordon 1959) and the measurement of radioactive I 131 in the faeces. Intestinal plasma protein loss is a non-specific finding associated with several diseases of the gastrointestinal tract. For example massive protein loss in the faeces may be caused by hypertrophic gastritis, gastric carcinoma, the sprue syndrome ulcerative colitis, salmonellosis, lymphomas obstruction of the thoracic duct, essential intestinal lymphangiectasia and a fall in venous pressure in the systemic circulation (Schwartz et al 1967). Plasma protein loss is also caused by certain general conditions accompanied by disturbances in capillary permeability such as shock radiation injuries and nephrosis (Barandum et al 1965). In view of these facts we decided to study the possible plasma protein loss in the faeces of toxæmic patients.

### Material and Method

Sixteen parturients were studied. Six had normal pregnancies four had preeclampsia of pregnancy and six had preeclampsia with toxæmia. A total of 44 faecal determinations were made. Each subject was given immediately post partum 15 drops of potas-

sium iodide three times a day for three days. She was then given 10  $\mu$ Ci of PVP I 131 by intravenous injection. Collection of faeces began after six hours or from the first subsequent defaecation and was continued for 48 hours. The specimens for each day were collected separately. Each specimen was homogenised and its radioactivity was measured and expressed as a percentage of the dose administered. Preliminary studies revealed that the maximum PVP I 131 excretion occurs within the period of 48 hours. The collection time varied from two to eight days (e.g. Gordon 1959, Jarnum 1961a). In the cases of toxæmia, a further specimen was collected 6–8 weeks *post partum*. Urinary protein excretion, urinary sedimentation and serum protein were also studied in relation to both periods of faecal collection.

### Results

The results are presented in its entirety in Table I. The excretion of PVP I 131 in the faeces of the six normal parturients (12 samples) immediately *post partum* was  $0.121 \pm 0.058$  per cent/24 hours. In the four parturients who had urinary tract infection with proteinuria (eight samples) it was  $0.088 \pm 0.042$  per cent/24 hours. There is no significant difference between these two groups. However the mean PVP I 131 excretion in the 24-hour faecal specimens (12 samples) collected from the six toxæmic patients immediately *post partum* was  $0.490 \pm 0.503$  and after 6–8 weeks it was  $0.322 \pm 0.240$  per cent/24 hours. The difference between the PVP I 131 excretion values of normal and toxæmic parturients immediately *post partum* is significant ( $0.01 \leq p \leq 0.05$ ). The difference is less marked ( $0.125 \leq p \leq 0.250$ ) when comparison is made between the faeces collected of 6–8 weeks from the patients with toxæmia. No distinct correlation with proteinuria is demonstrable. This emerges e.g. in Cases 14 and 16 where the excretion of proteinuria per 24 hours is the same but the corresponding PVP I 131 excretion in the faeces differs markedly. The serum protein values for toxæmic patients rose slightly 6–8 weeks after delivery. No hypoproteinemia was evident in any of the cases.

1 and 6-8 Weeks after Delivery (T xamale Patients)

Table 1 Results <i>Incomedia</i> by Full Partogram (200) Patients											
Case	Parity	Diagnosis	Results Immediately after Delivery				6-8 Weeks after Delivery				
			Bacteriuria		Sepsis Probable (%)	PVP-4-1311 in Faeces (%)		Bacteriuria	Sepsis Probable (%)	PVP-4-1311 in Faeces (%)	
			1	2		1	2				
1	I	Normal pregnancy	0	0	6.1	0	0	0.04	0.06		
2	I	Normal pregnancy	0	0	6.2	0	0	0.12	0.18		
3	I	Normal pregnancy	0	0	6.3	0	0	0.14	0.19		
4	I	Normal pregnancy	0	0	7.3	0	0	0.09	0.24		
5	IV	Normal pregnancy	0	0	7.1	0	0	0.15	0.06		
6	I	Normal pregnancy	0	0	7.0	0.1	0	0.15	0.04		
7	I	Pyelitis of pregnancy	0	0	6.0	0	0	0.01	0.10		
8	II	Pyelitis of pregnancy	+	1.0	5.8	1.0	1.0	0.13	0.16		
9	I	Pyelitis of pregnancy	0	0	6.0	1.0	0	0.02	0.10		
10	I	Pyelitis of pregnancy	0	0.1	6.0	0.9	0.1	0.11	0.08		
11	I	Pyelitis of pregnancy	0	2.4	6.3	4.0	2.4	0.34	0.42	0	0
12	I	Pre-eclampsia grade I	0	1.0	6.5	1.0	1.0	0.43	0.23	0	0
13	I	Pre-eclampsia grade I	0	2.7	6.4	2.7	2.3	0.27	0.30	0.1	0.1
14	I	Pre-eclampsia grade I	0	0.1	6.3	0.1	0.1	0.88	1.98	0	0
15	I	Pre-eclampsia grade I	0	2.1	7.2	2.1	2.5	0.16	0.59	0.1	0
16	I	Pre-eclampsia grade I	0	0.1	6.0	0.1	0.1	0.18	0.10	0	0
17	I	Pre-eclampsia grade I	0	0	6.8	0	0	0	0.41	0	0
18	I	Pre-eclampsia grade I	0	0	6.9	0	0	0	0.23	0	0
19	I	Pre-eclampsia grade I	0	0.1	7.5	0.1	0.1	0.27	0.35	0.1	0.1
20	I	Pre-eclampsia grade I	0	0	7.3	0	0	1.00	0.15	0	0
21	I	Pre-eclampsia grade I	0	0.1	7.0	0.1	0	0.04	0.29	0.1	0
22	I	Pre-eclampsia grade I	0	0	7.2	0	0	0	0.15	0	0
23	I	Pre-eclampsia grade I	0	0.1	6.0	0.1	0.1	0.18	0.10	0	0

Abbreviations: 1 = The first day 2 = The second day

sium iodide three times a day for three days. She was then given 10  $\mu$ Ci of PVP I 131 by intravenous injection. Collection of faeces began after six hours or from the first subsequent defaecation and was continued for 48 hours. The specimens for each day were collected separately. Each specimen was homogenised and its radioactivity was measured and expressed as a percentage of the dose administered. Preliminary studies revealed that the maximum PVP I 131 excretion occurs within the period of 48 hours. The collection time varied from two to eight days (e.g. Gordon 1959, Jarnum 1961a). In the cases of toxæmia, a further specimen was collected 6–8 weeks *post partum*. Urinary protein excretion, urinary sedimentation and serum protein were also studied in relation to both periods of faecal collection.

### Results

The results are presented in its entirety in Table I. The excretion of PVP I 131 in the faeces of the six normal parturients (12 samples) immediately *post partum* was  $0.121 \pm 0.058$  per cent/24 hours. In the four parturients who had urinary tract infection with proteinuria (eight samples) it was  $0.088 \pm 0.042$  per cent/24 hours. There is no significant difference between these two groups. However the mean PVP I 131 excretion in the 24-hour faecal specimens (12 samples) collected from the six toxæmic patients immediately *post partum* was  $0.490 \pm 0.503$  and after 6–8 weeks it was  $0.322 \pm 0.240$  per cent/24 hours. The difference between the PVP I 131 excretion values of normal and toxæmic parturients immediately *post partum* is significant ( $0.01 \leq p \leq 0.05$ ). The difference is less marked ( $0.125 \leq p \leq 0.250$ ) when comparison is made between the faeces collected of 6–8 weeks from the patients with toxæmia. No distinct correlation with proteinuria is demonstrable. This emerges e.g. in Cases 14 and 16 where the excretion of proteinuria per 24 hours is the same but the corresponding PVP I 131 excretion in the faeces differs markedly. The serum protein values for toxæmic patients rose slightly 6–8 weeks after delivery. No hypoproteinaemia was evident in any of the cases.

## SUMMARY

The PVP-I 131 faecal excretion test was performed on parturients immediately post partum. Faeces were collected separately for 48 hours. For twelve 24-hour samples collected from six normal parturients the PVP I 131 excretion value was  $1121 \pm 0.058$  per cent. It was  $0.088 \pm 0.042$  per cent/24 hours for four patients with pyelitis of pregnancy (eight samples). The value for six patients with toxæmia (12 samples) was  $0.490 \pm 0.503$  per cent/24 hours a statistically significant difference from the normal parturient value. Excretion decreased to  $0.322 \pm 0.240$  per cent after 6-8 weeks. The finding must be regarded as semiquantitative. The increase in plasma protein loss in the cases of toxæmia is probably due to alterations in permeability.

## REFERENCES

- Berendson, S., Kobler, H. and Diggelmann, H. *Bull. Schweiz. Akad. med. Wiss.* 21: 230, 1965.  
Carrere, D. and Lesko, L., *Ann. Chir. Gyn. Fenn.* 54: 443, 1965.  
Frundberg, U. and Lutz, J. *Arch. Gynäk.* 199: 96, 1963.  
Gordon, R. *Lancet* i: 325, 1959.  
Jernum, S. *Scand. J. Clin. Lab. Invest.* 13: 462, 1961.  
    *Ibidem* 13: 447, 1961b.  
Kukla, M. *Duodena* 82: 1023, 1966.  
Kohle, H. *Bull. Schweiz. Akad. med. Wiss.* 21: 298, 1965.  
Löhe, J., Leischke, H. J., Hartmann, H., Koch, H. and Seiger, K., *Rad. diagn.* 7: 771, 1966.  
Schwartz, K.-D., Knoll, P. and Kärner, K.-H. *Rad. tech. theor.* 8: 225, 1967.  
Waldman, T. and Lasser, L. *J. Clin. Invest.* 43: 1025, 1964.  
Wetterfors, A. *Acta med. scandav. Suppl.* 430: 177, 1963.

Received on May 11, 1968

### Discussion

The considerable protein loss that may occur through the gastrointestinal tract is associated with the active synthesis of digestive enzymes and the rapid catabolism of epithelial tissue. Plasma proteins occur normally in both gastric and intestinal juice and are digested like other proteins during the normal digestive processes. The catabolism of albumin occurs most rapidly in the duodenum (Wetterfors 1965)

The mucosa of the gastrointestinal tract lacks the "screening test" possessed by e.g. the renal glomeruli. The permeability of these glomeruli to protein fractions is dependent on the weight of the protein molecule even in an abnormal state (Alekki 1967). The molecular weight of PVP I 131 is c. 40 000 and it behaves like plasma protein unless the I 131 bond breaks. This is most likely to occur if the PVP preparation is old (Löbe *et al.* 1966). According to Jarnum (1961 b) faecal excretion of PVP in normal cases is 0-0.25 per cent/24 hours while in pathological cases it can even be 5 per cent/24 hours. The values for the toxæmic patients immediately *post partum* exceeded these normal values, whereas the results for the control and pyelitis still had higher excretion values than the controls after 6-8 weeks, but they were lower than the values immediately *post partum*.

The factors influencing albumin synthesis and catabolism are not precisely known. The maximum synthesising capacity of the liver is roughly three times the accepted normal (Kobler 1965). In contrast the body is capable of increasing many times the synthesis of gamma globulin. Hypogammaglobulinemia caused by accelerated catabolism is associated with e.g. the nephrotic syndrome which is also manifest clinically as protein loss via the kidneys and the intestines. This may be connected with changes in capillary permeability (Waldmann and Laster 1964). Corresponding changes in capillary permeability have been established in parturients (Friedberg and Lutz 1963). These are not, however, correlated with the degree of severity of toxæmia (Castrén and Laakso 1965) or was any correlation established in the present work with the 24-hour excretion of proteinuria. This reduction in capillary permeability in patients with toxæmia may also be the reason for the protein losing enteropathy.



## SUMMARY

The PVP I 131 faecal excretion test was performed on parturients immediately post partum. Faeces were collected separately for 48 hours. For twelve 24-hour samples collected from six normal parturients the PVP I 131 excretion value was  $0.121 \pm 0.058$  per cent. It was  $0.088 \pm 0.042$  per cent/24 hours for four patients with pyelitis of pregnancy (eight samples). The value for six patients with toxæmia (12 samples) was  $0.490 \pm 0.503$  per cent/24 hours, a statistically significant difference from the normal parturient value. Excretion decreased to  $0.322 \pm 0.240$  per cent after 6-8 weeks. The finding must be regarded as semiquantitative. The increase in plasma protein loss in the cases of toxæmia is probably due to alterations in permeability.

## REFERENCES

- Barandem, S. Kobler H. and Diggelmann, H. *Bull. Schweiz. Akad. med. Wiss.* 21 230 1965  
Castres, D. and Laakso, L. *Ann. Chir. Gyn. Fenn.* 54 443, 1965  
Friesberg, U. and Lutz, J. *Arch. Gynäk.* 199 96, 1963  
Gordon, R. *Lancet* i 325 1969  
Jernum, S. *Scand. J. Clin. Lab. Invest.* 13 462, 1961  
    *Ibidem* 13 447 1961 b  
Kukli, M. *Duodenum* 82, 1023 1966  
Kobler, H. *Bull. Schweiz. Akad. med. Wiss.* 21 298, 1965  
Lübke, J., Lerschke, H. J., Hartmann, H., Koch, H. and Seiger, K., *Rad. diagn.* 7 771 1966  
Schwartz, K. D., Knoll, P. and Kattner, K.-H. *Rad. biol. ultr.* 8 225, 1967  
Waldmann, T. and Lasser, L. *J. Clin. Invest.* 43 1025 1964  
Wettersfor, A. *Acta med. scandinav. Suppl.* 430 177 1963

Received on May 11 1968

### Discussion

The considerable protein loss that may occur through the gastrointestinal tract is associated with the active synthesis of digestive enzymes and the rapid catabolism of epithelial tissue. Plasma proteins occur normally in both gastric and intestinal juice and are digested like other proteins during the normal digestive processes. The catabolism of albumin occurs most rapidly in the duodenum (Wetterfors 1965).

The mucosa of the gastrointestinal tract lacks the screening test possessed by e.g. the renal glomeruli. The permeability of these glomeruli to protein fractions is dependent on the weight of the protein molecule even in an abnormal state (Lekki 1967). The molecular weight of PVP I 131 is c. 40 000 and it behaves like plasma protein unless the I 131 bond breaks. This is most likely to occur if the PVP preparation is old (Löbe *et al.* 1966). According to Jarnum (1961 b) faecal excretion of PVP in normal cases is 0-0.25 per cent/24 hours, while in pathological cases it can even be 5 per cent/24 hours. The values for the toxæmic patients immediately *post partum* exceeded these normal values, whereas the results for the control and pyelitis still had higher excretion values than the controls after 6-8 weeks but they were lower than the values immediately *post partum*.

The factors influencing albumin synthesis and catabolism are not precisely known. The maximum synthesising capacity of the liver is roughly three times the accepted normal (Lobler 1965). In contrast, the body is capable of increasing many times the synthesis of gamma globulin. Hypogammaglobulinemia caused by accelerated catabolism is associated with e.g. the nephrotic syndrome which is also manifest clinically as protein loss via the kidneys and the intestines. This may be connected with changes in capillary permeability (Waldmann and Laster 1964). Corresponding changes in capillary permeability have been established in parturients (Friedberg and Lutz 1963). These are not, however, correlated with the degree of severity of toxæmia (Castrén and Laakso 1965) or was any correlation established in the present work with the 24-hour excretion of proteinuria. This reduction in capillary permeability in patients with toxæmia may also be the reason for the protein losing enteropathy.

in the other because of foetal distress. The babies were all healthy and mature.

During labour the patients were all given nitrous oxide. Trichlorethylene or in a few cases chloroform was also administered at the end of the second stage of labour.

A non-pregnant control group consisted of fourteen healthy volunteers. The age distribution in the non-pregnant and the pregnant groups was similar.

## Methods

### 1 General procedure

In all subjects the carbon monoxide (CO) production, carboxy haemoglobin (COHb) saturation, haemoglobin (Hb) concentration and total amount of haemoglobin (THb) were measured.

In 20 of the pregnant women the measurements were made before and 0-24 hours after delivery and in 10 of these the external blood loss was measured as described by Robbe and Ström (1958) except that the blood was collected only for up to two hours post-partum. In the 13 remaining women no measurements were made after delivery due to Caesarian section, excessive bleeding, lack of cooperation etc.

CO-production was measured according to Linderholm (1968).

Total haemoglobin in the body (THb) was measured in conjunction with the measurement of CO production, either directly afterwards, or if the rebreathing procedure was using for the subject a few hours later. Most of the pregnant women preferred to be on their sides instead of in the usual semirecumbent position.

COHb saturation in blood was measured according to the alcolar CO method (Sjöström and 1948). A closed rebreathing system with O<sub>2</sub> and CO<sub>2</sub> absorption was used and the rebreathed gas was analysed.

For the CO analysis in gas three methods were used.

- 1 The palladium-molybdenum tube method (Andersson and Dahlström, 1958 and Dahlström, 1960)
- 2 The hopcalite method (Linderholm and Sjöstrand 1956)

From the Department of Clinical Physiology (Prof H Linderholm) and the Department of Obstetrics and Gynaecology (Prof P Lundström) University of Umeå Umeå Sweden

## ENDOGENOUS CARBON MONOXIDE PRODUCTION AND BLOOD LOSS AT DELIVERY

BY

H. LINDERHOLM AND P. LUNDSTRÖM

*Robbe Ström* and *Zetterström* (1960) who found a considerable increase in the percentage saturation of carboxyhaemoglobin in the blood two hours after delivery suggested that increased haemolysis might at least partially explain the "hidden blood loss" found to occur at delivery (*Löwenstein Pick* and *Philpott* 1950 *Verel Bury* and *Hope* 1956 and *Robbe* and *Ström* 1958). If increased haemolysis occurs this may be expected to give an increased production of carbon monoxide which it is now possible to measure (*Coburn Blakemore* and *Forster* 1963 *Linderholm* 1968). In order to investigate these problems the carbon monoxide production before and after delivery was studied.

### *Material*

Thirty three healthy pregnant women all non-smokers from a maternal welfare organization were included in the study. The pregnancy of these women was uneventful. For instance none had symptoms of toxæmia and none were Rh-iso-immunized. Three subjects had a moderate sideropenic anaemia.

Where post partum measurements were performed the delivery took place without or with only minor complications. No blood transfusion was given. In some cases episiotomy was carried out. In two cases vacuum extraction was performed, in one case owing to a slight prolongation of the second stage of labour and

Table 1. Comparison Between the Values of the COHb Saturation in Per Cent Obtained by Different Methods of Analysing the CO Content of the Re-breathed Gas

Period of Examination		Palladium-Molybdenum Tube Method	Hopcalite Method	Infra-red Method
Ante partum (1-10 hrs)	n	11	11	11
	M	0.44	1.20	6.61
	Md	0.45	0.47	0.61
	R	0.76-0.61	0.35-6.60	0.48->0.30
	S.D.	0.465	1.29	6.99
Post partum (0-2 hrs)	n	10	10	10
	M	0.64	1.02	10.39
	Md	0.60	0.71	8.55
	R	0.38-1.10	0.40-2.57	2.2-23.8
	S.D.	0.676	1.07	10.9
Post partum (2-8 hrs)	n	9	9	9
	M	0.67	0.62	9.00
	Md	0.60	0.53	3.10
	R	0.39-1.15	0.40-1.05	0.81-9.60
	S.D.	0.715	0.654	5.30
Post partum (8-24 hrs)	n	11	11	11
	M	0.61	0.58	1.08
	Md	0.57	0.58	0.82
	R	0.46-0.84	0.42-0.72	0.54-3.90
	S.D.	0.639	0.607	1.13

All the women, some of whom were smokers, were given  $N_2O$  during the last period of the second stage of labour the  $N_2O$  was supplemented with  $ClCl$  in 5 cases, and with  $CHCl_3$  in 2 cases. The dose and the duration of administration varied widely. Some subjects had received  $N_2O$  before the antepartum measurements were carried out.

During this period only  $N_2O$  was given.

Symbols: n number of cases, M mean, Md median, R range, S.D. standard deviation.

The postpartum values obtained with the hopcalite meter are similar to those found by Robbe Ström and Zetterström (1960).

The sensitivity of the infra red and the hopcalite meters to  $N_2O$  is the main cause for the discrepancy between the results

using a Stålex CO meter and a Philips potentiometer writer (OR 2210 A 21)

- 3 *The infra red absorption method* A Beckman LIB infrared analyser model 15 A was used in combination with a Philips potentiometer writer Dry gas was obtained by using a CaCl<sub>2</sub> filter

All methods were continuously checked by calibration with standard CO gas mixtures.

## 2. *The specificity of the methods for CO analysis*

None of the three methods used for analysis of CO in gas are entirely specific for CO Anaesthetic agents in particular may cause interference As the patients had inhaled nitrous oxide (N<sub>2</sub>O) and in some cases trichlorethylene (CHCl CCl<sub>2</sub>) or chloroform (CHCl<sub>3</sub>) the sensitivity of the analytical methods to these gases was examined.

It was found that the palladium molybdenum test tube method was quite insensitive to N<sub>2</sub>O CHCl<sub>3</sub> and CHCl CCl<sub>2</sub> even in high concentrations

The hopcalite meter was 50–100 times more sensitive to CO than to CHCl<sub>3</sub> and CHCl CCl<sub>2</sub> and about 2000 times more sensitive to CO than to N<sub>2</sub>O (concentrations in mols/litre)

When mixtures of N<sub>2</sub>O and CHCl<sub>3</sub> were analysed the deflection of the CO meter was less than the sum of the deflections caused by each gas separately

The infra red meter was about 10 times more sensitive to CO than to N<sub>2</sub>O CHCl<sub>3</sub> and CHCl CCl<sub>2</sub> did not produce any deflection when this meter was used.

The concentration of the anaesthetic gases may be expected to be high in relation to that of CO in expired air In order to study the effect on the analytical methods of the anaesthetics used 11 pregnant women were examined under conditions similar to those of the present study

Table I shows the erroneously high COHb saturation obtained when the results are based on CO analysis of the rebreathed gas with the hopcalite or the infra-red meter

Table 1. Comparison Between the Values of the COHb Saturation in Per Cent Obtained by Different Methods of Analysing the CO Content of the Re-breathed Gas

Period of Examination		Palladium-Molybdenum Tube Method	Hopcalite Method	Infra-red Method
Ant partum (1-10 hrs)	n	11	11	11
	M	0.44	1.20	6.61
	Md	0.45	0.47	0.61
	R	0.26-0.61	0.35-6.60	0.48- > 0.30
	S.D.	0.465	1.25	6.99
Post partum (0-2 hr)	n	10	10	10
	M	0.64	1.02	10.39
	Md	0.60	0.71	8.55
	R	0.39-1.10	0.40-2.57	2.2-23.8
	S.D.	0.676	1.07	10.9
Post partum (2-8 hrs)	n	9	9	9
	M	0.67	0.62	5.00
	Md	0.60	0.53	3.10
	R	0.39-1.15	0.40-1.05	0.81-9.60
	S.D.	0.715	0.654	5.30
Post partum (8-24 hrs)	n	11	11	11
	M	0.61	0.58	1.08
	Md	0.57	0.58	0.82
	R	0.46-0.84	0.42-0.72	0.54-3.90
	S.D.	0.639	0.607	1.13

All the women some of whom were smokers, were given N<sub>2</sub>O. During the last period of the second stage of labour the N<sub>2</sub>O was supplemented with C<sub>2</sub>H<sub>6</sub> in 5 cases, and with Cl<sub>2</sub>CCl<sub>2</sub> in 2 cases. The dose and the duration of administration varied widely. Some subjects had received N<sub>2</sub>O before the antepartum examinations were carried out.

During this period only N<sub>2</sub>O was given.

Symbols: n number of cases, M mean, Md median, R range, S.D. standard deviation.

The postpartum values obtained with the hopcalite meter are similar to those found by Robbe Ström and Zetterström (1960).

The sensitivity of the infra red and the hopcalite meters to N<sub>2</sub>O is the main cause for the discrepancy between the results

obtained with the palladium-molybdenum method on the one hand and the hopcalite and infra red methods on the other

It was found that even before  $N_2O$  was administered, the presence of  $N_2O$  in the air of the delivery room was sufficient to affect the results of the analysis with the hopcalite and infra-red meters.

In addition  $CHCl_3$ , and probably even  $CHCl CCl_2$ , contributed to the erroneously high COHb concentration obtained with the hopcalite method in the period 0-2 hours after delivery

In some cases the COHb saturation of the blood was directly determined with the palladium-molybdenum tube micromethod (Linderholm 1965) and with the method of Linderholm Sjöstrand and Söderström (1966). The samples were taken before and after delivery at the end of the rebreathing procedure. The results which are not accounted for in the tables agreed well with those obtained with the alveolar method using the palladium-molybdenum tube method for CO analysis but not with those obtained with the hopcalite and infra red methods.

Unless filters are used the hopcalite method is unsuitable for CO analysis if  $N_2O$  or  $CHCl_3$  (and probably  $CHCl CCl_2$ ) is present in the respiratory gas. The same is true for the infra red meter as far as  $N_2O$  is concerned. Therefore all figures under Results are based on CO analysis with the palladium-molybdenum test tube method.

### Results

According to Table II neither the Hb concentration nor the COHb saturation changed appreciably up to 24 hours after delivery. The mean CO production seemed to be almost the same before and after delivery even when the calculations were based on the individual differences. The mean CO production was somewhat lower in the control group than in the group of pregnant women while the rate of CO production per g THb was approximately the same in both groups. Calculated from the individual differences the mean CO production per hour per g THb before delivery and up to 24 hours after delivery showed rather small and statistically insignificant changes.



Table II Haemoglobin Concentration, Total Amount of Haemoglobin (THb), Saturation of Hb with CO (COHb), Total CO Production, and CO Production per g THb in Pregnant Women before and 0-24 Hours after Delivery and in a Control Group of Non-pregnant Women

	n	Hb Conc. g/100 ml	THb g	COHb per cent	CO Produc- tion ml/hr	CO Produc- tion $\mu$ l/hr/g THb
Mean and standard deviation						
Non-pregnant controls	14	$10.7 \pm 0.85$	$460 \pm 76$	$0.44 \pm 0.11$	$0.39 \pm 0.23$	$0.90 \pm 0.51$
Pregnant women						
Ant. partum	33	$10.5 \pm 2.3$	$622 \pm 119$	$0.40 \pm 0.11$	$0.59 \pm 0.38$	$0.88 \pm 0.61$
0-2 hr pp	9	$11.6 \pm 1.2$	$564 \pm 117$	$0.36 \pm 0.09$	$0.58 \pm 0.20$	$1.06 \pm 0.44$
0-4 hr pp	13	$11.9 \pm 1.0$	$566 \pm 113$	$0.35 \pm 0.09$	$0.60 \pm 0.19$	$1.10 \pm 0.39$
4-24 hrs pp	14	$11.1 \pm 1.2$	$541 \pm 106$	$0.38 \pm 0.11$	$0.67 \pm 0.32$	$1.22 \pm 0.43$
Mean and standard deviation of the individual differences						
0-2 hr pp	n					
0-2 hr pp	9	$0.32 \pm 0.96$	$47 \pm 92$	$0.04 \pm 0.11$	$-0.13 \pm 0.47$	$0.06 \pm 0.71$
0-4 hr pp	13	$0.16 \pm 0.83$	$70 \pm 101$	$-0.07 \pm 0.14$	$0.08 \pm 0.49$	$+0.07 \pm 0.72$
4-24 hrs pp	14	$0.01 \pm 1.20$	$71 \pm 73$	$0.06 \pm 0.14$	$+0.09 \pm 0.51$	$0.23 \pm 0.86$
0-4 hrs pp	20	$0.30 \pm 0.99$	$66 \pm 87$	$-0.04 \pm 0.13$	$\pm 0.00 \pm 0.49$	$0.11 \pm 0.76$
pp	post partum		p	ante partum		

Table III Total Amount of Haemoglobin (THb) ante and post partum, and 1st real Blood Loss at Delivery ( $\pm 10$ )

	Mean	Range
THb g, 2-18 day ante partum	641	495-770
THb g, 3-5 day post partum	534	450-622
Total Hb loss (difference THb ante-post partum)	108	10-173
External Hb loss g, up to 2 hours post partum	30	15-56
Difference between total and estimated external Hb loss g <sup>a</sup>	42 <sup>a</sup>	35-106

The estimated external blood loss was calculated in the following way: the mean loss of 30 g of Hb up to 2 hours post partum  $\times$  7 g (2 hours post partum  $\times$  4 day post partum) 14 g (placental loss) and 13 g (=fetal loss) were added in, using to Robbe and Sjödin (1948)

<sup>a</sup> 0.01

The total amount of haemoglobin (THb) in the 10 subjects in whom the external blood loss at delivery was measured was on the average 106 g less 3-5 days after delivery than before delivery. At delivery and during the first 4 post partum days the estimated average external blood loss was 64.6 g Hb. The difference is  $42.2 \pm 14.6$  g Hb ( $P < 0.01$ ).

### Discussion

#### CO production

The COHb saturation reflects the rate of CO production but it is also influenced by such factors as the CO concentration in ambient air and the alveolar ventilation (Coburn *et al.* 1963). During pregnancy and labour hyperventilation and a high  $O_2$  consumption is present (Cugell, Frank and Gaensler 1953; Cohen and Thomson 1939). After delivery  $O_2$  consumption and ventilation decrease. Therefore the rate of CO production is best measured directly.

In our series the mean CO production of the pregnant women, 0.59 ml CO per hour, was somewhat higher than that of the healthy non pregnant controls, 0.39 ml CO per hour (Coburn *et al.* 1963). Using a somewhat different method, found the mean production in a group of 10 young men to be 0.42 ml CO per hour.

The CO produced by the body originates mainly from the breakdown of Hb (Coburn *et al.* 1964; Luomanmäki 1966). It was therefore of interest to study the rate of CO production per g THb. As this was approximately equal in pregnant women during the last month of pregnancy and in non pregnant women it seems likely that the increase in THb during pregnancy accounts for most of the increase of the CO production rate. The CO production per g THb is higher in our groups than that reported by Coburn, Forster and Kane (1965). The difference is probably due to methodological dissimilarities.

After delivery the rate of CO production did not change significantly in spite of the decrease in THb. This may indicate a relatively high rate of CO production during the first 24 hours after delivery that may be related to lesions of the parturient canal and to degeneration of the decidual tissue. An increase of

COHb concentration indicating increased haemolysis after surgery has been described (Ibring, 1958/59)

#### COHb saturation

According to Table II the COHb saturation before and after delivery was unchanged, but in Table I an increase after delivery is found. This statistically insignificant increase was due to a high COHb concentration in two subjects who were smokers. Our results differ from those obtained by Robbe *et al* (1960) who found a 240 per cent increase in the COHb saturation two hours after delivery. This may be due to an analytical error as the hopcalite apparatus they used is sensitive to anaesthetic gases (see under Methods).

*The loss of haemoglobin at and after delivery* In our investigation the THb was considerably lower 3-5 days after delivery than before delivery. This decrease in THb exceeds the estimated external loss of Hb at and after delivery (see footnote Table III). This is in agreement with the findings of Löwenstein *et al* (1950), Verel *et al* (1956) and Robbe and Ström (1958).

Assuming that the haemolysis of 1 g of Hb results in the formation of 1.34 ml of CO, 40-55 ml CO will be produced by the haemolysis of 30-40 g Hb. As a "hidden" loss of this magnitude seems to take place at or immediately after delivery, an increased COHb saturation would without doubt be found if the loss in question depended to any great extent on haemolysis. We found no such increase. In addition, the change in the rate of CO production was negligible. The hypothesis that the "hidden blood loss" after delivery is partly due to haemolysis is not sustained by our investigation. For the time being we can offer no explanation of the fall in THb at delivery and during the first four postpartum days.

#### SUMMARY

The rate of CO production was determined in 33 pregnant women during the last month of pregnancy and during the first 24 hours after delivery and in 14 healthy non-pregnant controls. It was approximately the same in the pregnant and non-pregnant women.

The total amount of haemoglobin (THb) in the 10 subjects in whom the external blood loss at delivery was measured, was on the average 106 g less 3-5 days after delivery than before delivery. At delivery and during the first 4 post partum days the estimated average external blood loss was 64.6 g Hb. The difference is  $42.2 \pm 14.6$  g Hb ( $P < 0.01$ ).

### Discussion

#### CO production

The COHb saturation reflects the rate of CO production but it is also influenced by such factors as the CO concentration in ambient air and the alveolar ventilation (Coburn *et al.* 1963). During pregnancy and labour hyperventilation and a high  $O_2$  consumption is present (Cugell, Frank and Gaensler 1953; Cohen and Thomson 1939). After delivery  $O_2$  consumption and ventilation decrease. Therefore the rate of CO production is best measured directly.

In our series the mean CO production of the pregnant women 0.59 ml CO per hour was somewhat higher than that of the healthy non-pregnant controls 0.39 ml CO per hour (Coburn *et al.* 1963) using a somewhat different method found the mean production in a group of 10 young men to be 0.42 ml CO per hour.

The CO produced by the body originates mainly from the breakdown of Hb (Coburn *et al.* 1964; Luomanmäki 1966). It was therefore of interest to study the rate of CO production per g THb. As this was approximately equal in pregnant women during the last month of pregnancy and in non-pregnant women it seems likely that the increase in THb during pregnancy accounts for most of the increase of the CO production rate. The CO production per g THb is higher in our groups than that reported by Coburn, Forster and Kane (1965). The difference is probably due to methodological dissimilarities.

After delivery the rate of CO production did not change significantly in spite of the decrease in THb. This may indicate a relatively high rate of CO production during the first 24 hours after delivery that may be related to lesions of the parturient canal and to degeneration of the decidual tissue. An increase of

## COAGULATION AND FIBRINOLYSIS IN NORMAL WOMEN IMMEDIATELY POST PARTUM AND IN NEWBORN INFANTS

Influence of Prophylactic Vitamin K

BY

NIELS CHR. NIELSEN

A number of studies have been concerned with coagulation and the fibrinolytic system in the parturient woman and the newborn infant. However the majority of these studies have included only a very few patients or a few coagulation and fibrinolysis parameters. There has been no major collected study on coagulation as well as fibrinolysis in mothers and their newborn infants.

### *Previous Investigations*

#### *First Phase of Coagulation*

**Platelet** The platelet count in the peripheral maternal blood immediately after delivery is often reported to be at the lower end of the normal range Bellwinkel (1951) and Nilsen (1963) have reviewed the early literature. The findings have later been confirmed by Skjold and Albrechtsen (1965).

In cord blood the platelet count is approximately the same as in adults (Sanford and Shnitgelsky 1942 Larrieu *et al.* 1952 Runge *et al.* 1954 and others). Runge *et al.* demonstrated that there is no relationship between the counts in the mother and her infant.

**Factor VIII (Antihæmophilic factor AHP)** The content is

and did not change significantly after delivery. In some of the pregnant women the total haemoglobin of the body (THb) was determined before and after delivery as well as the external blood loss during and after delivery. The decrease in THb was greater than the estimated external loss of haemoglobin. The "hidden" blood loss could not be accounted for even partly by increased haemolysis.

An investigation of the specificity of the methods for CO analysis revealed that the palladium molybdenum test tube method for analysis of CO in gas was insensitive to nitrous oxide, trichloroethylene and chloroform. The hopcalite meter was sensitive to nitrous oxide, chloroform and probably to trichloroethylene and the infra red meter to nitrous oxide.

#### REFERENCES

- Andersson T and Dahlström H. *Science Tools* 5:1 1958.  
 Coburn R. F., Blakemore W. S. and Forster R. E. *J. clin. Invest.* 42: 1172, 1963.  
 Coburn R. F., Forster R. E. and Lane P. B. *J. clin. Invest.* 44: 1899 1965.  
 Coburn R. F., Williams W. J. and Forster R. E. *J. clin. Invest.* 43: 1098, 1964.  
 Cohen M. E. and Thomson A. J. *J.A.M.A.* 112: 1556 1939.  
 Cugell D. W., Frank N. R., Gaensler E. A. and Badger T. C. *Am. Rev. Tuberc.* 67: 568 1953.  
 Dahlström H. *Scand. J. Clin. Lab. Invest.* 12: 39b 1960.  
 Ibring, G. *Acta Chir. Scand.* 116: 79 1958/59.  
 Linderholm H. *Acta Physiol. Scand.* 64: 372 1965.  
 Linderholm H. to be published 1968.  
 Linderholm H. and Sjöstrand T. *Acta Physiol. Scand.* 37: 240 1956.  
 Linderholm H., Sjöstrand T. and Söderström B. *Acta Physiol. Scand.* 66: 1 1966.  
 Luomanmaki A. *Am. Med. Exper. t Biol. Fenniae Suppl.* 2, 1966.  
 Löwenstein L., Pick C. A. and Philpott N. W. *Am. J. Obst. Gynec.* 60: 1206 1950.  
 Robbe H. and Ström G. *Acta Obst. et Gynec. Scand.* 37: 448 1958.  
 Robbe H., Ström G. and Zetterström B. *Nordiska gynekologkongressens förhandl.* Augusti 1960.  
 Sjöstrand T. *Acta Physiol. Scand.* 16: 211 1948.  
 Verel D., Bury J. D. and Hope A. *Clin. Sci.* 15: 1 1956.

## COAGULATION AND FIBRINOLYSIS IN NORMAL WOMEN IMMEDIATELY POST PARTUM AND IN NEWBORN INFANTS

*Influence of Prophylactic Vitamin K*

BY

NIELS CHR. NIELSEN

A number of studies have been concerned with coagulation and the fibrinolytic system in the parturient woman and the newborn infant. However the majority of these studies have included only a very few patients or a few coagulation and fibrinolysis parameters. There has been no major collected study on coagulation as well as fibrinolysis in mothers and their newborn infants.

### *Previous Investigations*

#### *First Phase of Coagulation*

**Platelets.** The platelet count in the peripheral maternal blood immediately after delivery is often reported to be at the lower end of the normal range Bellwinkel (1951) and Nilsen (1963) have reviewed the early literature. The findings have later been confirmed by Skjold and Albrechtsen (1965).

In cord blood the platelet count is approximately the same as in adults (Sanford and Shmigel'sky 1942 Larrieu *et al.* 1952 Runge *et al.* 1954 and others). Runge *et al.* demonstrated that there is no relationship between the counts in the mother and her infant.

**Factor VIII (Antihæmophilic factor AHF)** The content is

increased in parturient women and normal in cord blood. This was confirmed by *Kasper et al* (1964) who reviewed previous publications.

*Factor IX* (Christmas factor) is increased in maternal blood (*Fresh et al* 1956 *Rarnoff and Holland* 1959 and *Kasper et al* 1964) Other authors *Nilsson* and *Kullander* (1967 a) using haemophilic B plasma as test substrate found small or no increase. The cord blood on the other hand shows a fairly pronounced decrease (*Fresh et al* 1956 *Barlhan* 1957 and *Künzer and Ströder* 1957)

### *Second Phase of Coagulation*

*Factor II* (Prothrombin) For reviews of the literature and assessment of the various methods for prothrombin determination the reader is referred to *Thordarson* (1941) *Dyggve* (1952) *Larsen* (1952) *Schwen et al* (1958) *Nilson* (1963) and *Nilsson and Kullander* (1967 a)

In the light of previous reports it may be said that the prothrombin content is either elevated or possibly only slightly affected in the maternal blood and decreased in cord blood when the determinations are carried out by a two-stage technique or by a specific one-stage technique

It is agreed that no relationship is to be found between the prothrombin content of the maternal blood at delivery and the umbilical cord blood *Dyggve* (1952) has reviewed the literature on this aspect.

*Factor VII* (Proconvertin) The content in the maternal peripheral blood in late pregnancy and at parturition is elevated. A number of reports have been reviewed by *Nilsen* (1963)

*Dyggve* (1958) referred to a number of studies all of which showed a reduced proconvertin content in cord blood and demonstrated that no relationship exists between the proconvertin content of the maternal blood and the infant's blood.

*Factor X* (Stuart Prower factor) is increased in the blood of pregnant women (*Pechet and Alexander* 1961) whereas in cord blood it is reduced (*Beller* 1957 and *Kün et al* 1964)

*Factor V* (Proaccelerin) In late pregnancy and at delivery the



values are generally considered to be normal (Larrieu *et al.*, 1952 Alexander *et al.*, 1956 Fresh *et al.*, 1956 Beller 1957 Schwanzer *et al.*, 1958 Nilsen, 1963 Skjott and Albrechtsen, 1965 and Nilsson and Kullander 1967 a) However in a small series Schulze and Schwick (1953 a) demonstrated an increase in factor V levels.

In cord blood the content of factor V is usually found to be normal (Larrieu *et al.* 1952 Quick *et al.* 1952 Schulze and Schwick 1953 a Beller 1957 Forgács *et al.*, 1962, and others) However Fresh *et al.* (1956) and Vest and Meir (1957) have reported an increased content.

In a few cases where maternal values were elevated, the content in the cord blood was also elevated (Beller 1957)

### Third Phase of Coagulation

**Factor I (Fibrinogen)** As early as 1885 it was observed that the fibrinogen content of cord blood was considerably lower than that of the maternal blood (Krüger 1886) This observation has been confirmed by a large number of investigations.

Nilsen (1963) reviewed a number of publications reporting increased fibrinogen in the blood during pregnancy

In newborn infants the fibrinogen content is usually at the lower end of the normal range Taylor (1957) reviewed a number of early papers and these findings were later confirmed by Engström and Lager (1964) Markarian *et al.* (1967) and others.

**Haematocrit** Most authors have reported a slightly decreasing haematocrit towards term and an increase *post partum*. Some of the early publications have been reviewed by Nilsen (1963)

Cord blood has a higher haematocrit level than adult blood (Mugrage and Andersen 1936 Waugh *et al.* 1939 and Gairdner *et al.* 1952)

### Fibrinolysis

The different and often conflicting results published on the fibrinolytic components during pregnancy and delivery as well as in newborn infants are due largely to varying test methods and the lability of the active enzyme

increased in parturient women and normal in cord blood. This was confirmed by *Kasper et al* (1964) who reviewed previous publications.

*Factor IX* (Christmas factor) is increased in maternal blood (*Fresh et al* 1956 *Ratnoff and Holland* 1959 and *Kasper et al* 1964) Other authors *Nilsson* and *Kullander* (1967a) using haemophilic B plasma as test substrate found small or no increase. The cord blood, on the other hand shows a fairly pronounced decrease (*Fresh et al* 1956 *Barkhan* 1957 and *Kun er and Ströder* 1957)

### *Second Phase of Coagulation*

*Factor II* (Prothrombin) For reviews of the literature and assessment of the various methods for prothrombin determination the reader is referred to *Thordarson* (1941) *Dyggve* (1952) *Larsen* (1952) *Schwen er et al* (1958) *Nilson* (1963) and *Nilsson and Kullander* (1967a)

In the light of previous reports it may be said that the prothrombin content is either elevated or possibly only slightly affected in the maternal blood and decreased in cord blood when the determinations are carried out by a two-stage technique or by a specific one-stage technique

It is agreed that no relationship is to be found between the prothrombin content of the maternal blood at delivery and the umbilical cord blood. *Dyggva* (1952) has reviewed the literature on this aspect.

*Factor VII* (Proconvertin) The content in the maternal peripheral blood in late pregnancy and at parturition is elevated. A number of reports have been reviewed by *Nilsen* (1963)

*Dyggve* (1958) referred to a number of studies all of which showed a reduced proconvertin content in cord blood and demonstrated that no relationship exists between the proconvertin content of the maternal blood and the infant's blood.

*Factor X* (Stuart Prower factor) is increased in the blood of pregnant women (*Pechet and Alexander* 1961) whereas in cord blood it is reduced (*Beller* 1957 and *Künzer et al* 1964)

*Factor V* (Proaccelerin) In late pregnancy and at delivery the

values are generally considered to be normal (Larrieu *et al.*, 1952 Alexander *et al.* 1956 Fresh *et al.*, 1956 Beller 1957 Schwenzer *et al.*, 1958 Nilssen 1963 Skjold and Albrechtson, 1965 and Nilsson and Kullander 1967 a) However in a small series Schulze and Schwick (1953 a) demonstrated an increase in factor V levels.

In cord blood the content of factor V is usually found to be normal (Larrieu *et al.* 1952 Quick *et al.* 1952 Schulze and Schwick, 1953 a Beller 1957 Forgas *et al.*, 1962, and others) However Fresh *et al.* (1956) and Vess and Meir (1957) have reported an increased content.

In a few cases where maternal values were elevated, the content in the cord blood was also elevated (Beller 1957)

### Third Phase of Coagulation

**Factor I (Fibrinogen)** As early as 1885 it was observed that the fibrinogen content of cord blood was considerably lower than that of the maternal blood (Krüger 1886) This observation has been confirmed by a large number of investigations.

Nilssen (1963) reviewed a number of publications reporting increased fibrinogen in the blood during pregnancy

In newborn infants the fibrinogen content is usually at the lower end of the normal range Taylor (1957) reviewed a number of early papers and these findings were later confirmed by Engstrom and Kager (1964) Markarian *et al.* (1967) and others.

**Haematocrit** Most authors have reported a slightly decreasing haematocrit towards term and an increase post partum. Some of the early publications have been reviewed by Nilssen (1963)

Cord blood has a higher haematocrit level than adult blood (Mugrage and Andersen 1936 Waugh *et al.* 1939 and Gairdner *et al.* 1952)

### Fibrinolysis

The different and often conflicting results published on the fibrinolytic components during pregnancy and delivery as well as in newborn infants are due largely to varying test methods and the lability of the active enzyme

A large number of studies have revealed a reduced fibrinolytic activity in healthy pregnant women. On the other hand, the activity is increased immediately post partum. Nielsen (1963) reviewed the literature, but in his own study he could not demonstrate increased fibrinolytic activity in plasma either during or after delivery but in the euglobulin fraction a slight or moderate increase was found. In a recent study Nilsson and Kullander (1967a) were not able to demonstrate any increase in the fibrinolytic activity in plasma immediately before and after delivery.

The plasminogen content has been mentioned by only a few authors. Shaper *et al.* (1965) and Brakman (1966) reported unchanged values during the pregnancy and puerperium, while Samartzis *et al.* (1960) Skjær and Albrichsen (1965) Nilsson and Kullander (1967a) and others, stated that the plasminogen content was elevated at the time of delivery.

In cord blood fibrinolytic activity is increased (Berglund (1958) Phillips and Skrodzlis (1958) Ambrus *et al.* (1963) Beller *et al.* (1966) Engström and Kager (1964) and others) Engström and Kager reviewed the literature. In the studies marked the fibrinolytic activity in mother and infant was studied simultaneously but no direct relationship was shown.

In cord blood the plasminogen level is reduced (Cope and Mitchell 1964 Baller *et al.* 1966 and others)

### *Influence of Vitamin K upon the Clotting and Fibrinolytic Factors*

As far as the mothers are concerned, only prothrombin has been studied. Bohlender *et al.* (1941) and McCready *et al.* (1942) could not demonstrate any effect of vitamin K, while Quick *et al.* (1952) found longer prothrombin times in parturient women who had not been treated with vitamin K than among those who had.

Cord blood has been studied in respect to factors II, V and VII.

There is great disagreement concerning the influence of vitamin K, both as far as prothrombin and proconvertin are concerned.

Dyggve (1958) has given an extensive review of the literature on this subject.

Factor V is not affected by vitamin K (Quick *et al.*, 1952, and Fresh *et al.* 1957)

There has been no investigations on the influence of vitamin K upon fibrinolysis.

### *Present Investigations*

#### *Material and Methods*

Analyses were performed on blood samples from the following three groups

**Group 1** Twenty-five normal, non-pregnant women in the age range 19 to 38 years, average 24 years. The blood samples were drawn between 8 and 9 a.m. regardless of the relation to menstrual cycle since this has been shown by Beller *et al.* (1964) and Bralman *et al.* (1966) to have no influence upon coagulation or fibrinolysis. It was ensured that none of these women was using hormonal contraceptives which may give rise to changes in these coagulation components (Amris and Starup 1967 Bralman *et al.* 1967 Nilsson and Kullander 1967 b and others)

This group of normal women will be used below as a basis for comparison of coagulation and fibrinolysis in normal women immediately post partum and in newborn infants.

**Group 2** Twenty normal women in whom the samples were drawn within the first half-hour after normal delivery. None of these women had received vitamin K.

Blood samples were obtained also from these women's newborn infants within the first 20 min. after birth. All the infants were full-term, healthy and showed no abnormalities during their stay in the department.

**Group 3.** Twenty normal women and their infants. This group of women received menadione tablets, either 20 mg daily or 40 mg as a single dose between 2 and 24 hours before delivery. The results showed no difference between the two dosage schedules.

The blood samples from the adults were drawn from a cubital vein after application of a light tourniquet. The vein was punctured with a siliconized disposable needle and the samples were

A large number of studies have revealed a reduced fibrinolytic activity in healthy pregnant women. On the other hand, the activity is increased immediately *post partum*. Nielsen (1963) reviewed the literature but in his own study he could not demonstrate increased fibrinolytic activity in plasma either during or after delivery but in the euglobulin fraction a slight or moderate increase was found. In a recent study Nilsson and Kullander (1967 a) were not able to demonstrate any increase in the fibrinolytic activity in plasma immediately before and after delivery.

The plasminogen content has been mentioned by only a few authors. Shaper *et al* (1965) and Brakman (1966) reported unchanged values during the pregnancy and puerperium, while Samart *is et al* (1960) Skjods and Albrechtsen (1965), Nilsson and Kullander (1967 a) and others stated that the plasminogen content was elevated at the time of delivery.

In cord blood fibrinolytic activity is increased (Berglund (1958) Phillips and Skrodets (1958) Ambrus *et al.* (1963) Beller *et al* (1966) Engstrom and Kager (1964) and others) Engström and Kager reviewed the literature. In the studies marked the fibrinolytic activity in mother and infant was studied simultaneously but no direct relationship was shown.

In cord blood the plasminogen level is reduced (Cope and Mitchell 1964 Beller *et al* 1966 and others)

### *Influence of Vitamin K upon the Clotting and Fibrinolytic Factors*

As far as the mothers are concerned, only prothrombin has been studied. Bohlender *et al.* (1941) and McCreedy *et al* (1942) could not demonstrate any effect of vitamin K, while Quick *et al* (1952) found longer prothrombin times in parturient women who had not been treated with vitamin K than among those who had.

Cord blood has been studied in respect to factors II V and VII

There is great disagreement concerning the influence of vitamin K, both as far as prothrombin and proconvertin are concerned

Dyggve (1958) has given an extensive review of the literature on this subject.

Factor V is not affected by vitamin K (Quick *et al.*, 1952, and Fresh *et al.* 1957)

There have been no investigations on the influence of vitamin K upon fibrinolysis.

### *Present Investigations*

#### *Material and Methods*

Analyses were performed on blood samples from the following three groups

Group 1 Twenty-five normal non-pregnant women in the age range 19 to 38 years, average 24 years. The blood samples were drawn between 8 and 9 a.m. regardless of the relation to menstrual cycle since this has been shown by Beller *et al.* (1964) and Brakman *et al.* (1966) to have no influence upon coagulation or fibrinolysis. It was ensured that none of these women was using hormonal contraceptives which may give rise to changes in these coagulation components (Amris and Starup 1967 Brakman *et al.* 1967 Nilsson and Kullander 1967 b and others)

This group of normal women will be used below as a basis for comparison of coagulation and fibrinolysis in normal women immediately post partum and in newborn infants.

Group 2 Twenty normal women in whom the samples were drawn within the first half hour after normal delivery. None of these women had received vitamin K.

Blood samples were obtained also from these women's newborn infants within the first 20 min. after birth. All the infants were full-term, healthy and showed no abnormalities during their stay in the department.

Group 3 Twenty normal women and their infants. This group of women received menadione tablets, either 20 mg daily or 40 mg as a single dose between 2 and 24 hours before delivery. The results showed no difference between the two dosage schedules.

The blood samples from the adults were drawn from a cubital vein after application of a light tourniquet. The vein was punctured with a siliconized disposable needle, and the samples were

used only if the puncture proved easy. The blood was collected into ordinary glass tubes (14×100 mm) and into Owren's plastic thrombotest tubes. As anticoagulant sodium citrate 3.8% was used at the ratio 1:10 except in the haematocrit determinations where dried potassium ammonium oxalate was employed.

Blood collected into plastic tubes was centrifuged immediately at 1500 r.p.m. for 5 min. (platelet rich plasma) and analysed immediately.

Blood collected into glass tubes was centrifuged for 30 min. at 3500 r.p.m. (platelet-poor plasma) whereupon the plasma was frozen and stored at -20 °C for later analysis.

From the newborn infants blood was drawn by the following technique. Immediately after birth the cord was cut after application of *Hesseltine's* clamp (1937) about 5 cm from the skin. The cord was incised and a disposable plastic catheter was passed to the umbilical vein. The sample was used only if there was a free flow through the catheter.

The analyses stated below were performed using the platelet rich plasma for item (1) and (2) and the platelet poor plasma for the rest.

All the results except those of the fibrinolytic activity and the thromboplastin activation test, are given as the mean of two analyses. The fibrinolysis values are the mean of three determinations.

(1) Recalcification time in sec., in a mixture of 2 ml veronal buffer 0.25 M 0.025 M  $\text{CaCl}_2$  and 0.25 ml plasma, performed in a Owren thrombotest plastic tube.

(2) Thromboplastin activation test (two-stage TAT) as a modified *Ollendorff and Astrup* procedure (1966) using plastic instead of siliconized glass.

This investigation concerns the formation of plasma thromboplastin and thrombin in the plasma following recalcification. The result is shown in a diagram in which the abscissa is the incubation period in min. and the ordinate the log of the clotting time in sec. This diagram is characterized by the following values:  $t_1$ ,  $t_7$ ,  $T_{\text{max}}$  and  $t_{\text{max}}$  as well as the recalcification time (cf Fig 1). Combined with the other coagulation analyses the two-stage TAT tells whether the thromboplastin activity is increased. However this particular factor is better elucidated by a modification of the test viz three-stage TAT.

In principle the three-stage TAT serves to demonstrate any cl-t-promoting components in the test plasma by determining its coagulation-activating effect upon normal platelet-rich human plasma.



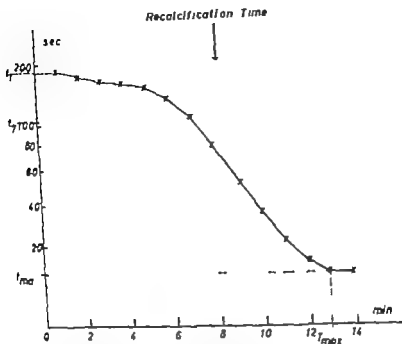


Fig 1 Thromboplastin activation curve of peripheral blood from 25 normal women. The curve is characterized by the following values:

- 1 Recalcification time in the incubation mixture in seconds (marked by arrow)
- 2  $t$  Coagulation time in the first tube, one minute after recalcification of the incubation mixture.
- 3  $t_7$  Coagulation time in the seventh tube 7 minutes after recalcification of the incubation mixture.
- 4  $T_{max}$  The number of minutes until the shortest clotting time is obtained.
- 5  $t_{max}$  The clotting time in seconds at  $T_{max}$ .

The incubation mixture consisted of 2.0 ml veronal buffer + 0.20 ml normal platelet-rich human plasma + 0.05 ml platelet-rich test plasma + 0.25 ml 0.025 M  $\text{CaCl}_2$ .

(3) Partial thromboplastin time (PTT) in sec according to the method described by Nye *et al* (1962). Thromboplastin Ortho® was used as the partial thromboplastin reagent.

(4) Thrombin time by determination of the clotting time in sec in a mixture of 0.1 ml plasma, 0.1 ml veronal buffer and 0.1 ml thrombin solution (20 NIH/ml) at 37° C.

(5) Prothrombin time in sec by the method of Quick (1935).

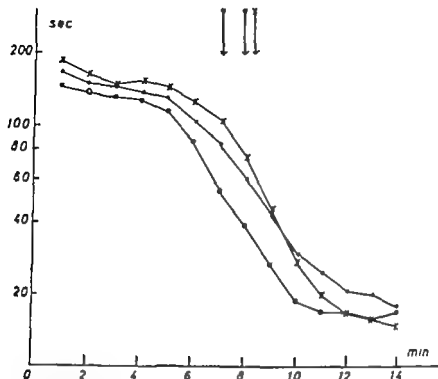


Fig. 2. Thromboplastin activation curve of peripheral blood from 20 normal women immediately after delivery. None had received vitamin K.

x—x 15 normal adult women, two-stage TAT

●—● 20 normal women immediately after delivery two-stage TAT

○—○ 20 normal women immediately after delivery three-stage TAT

(6) Factor V measured in per cent of normal as described by Biggs and Macfarlane (1965)

(7) Prothrombin-proconvertin in per cent of normal (PP) by the method of Owren and As (1951)

(8) Fibrinogen measured in mg/100 ml citrated plasma as advocated by Sjøvold and Albrechtsen (1965). The result was corrected for the dilution with sodium citrate

(9) Plasminogen measured in  $\mu$ g copper tyrosine released per ml activated plasma (Albjærgsø et al 1959)

(10) Fibrinolytic activity determined on standard fibrin plates (Astrup and Møller 1952) and on heated fibrin plates (Lassen 1952). The plates were produced from bovine fibrinogen 0.2% Pov-te®

Every heated plate was checked by the plasminogen activator urokinase to make sure that all the plasminogen had been destroyed by the heating.

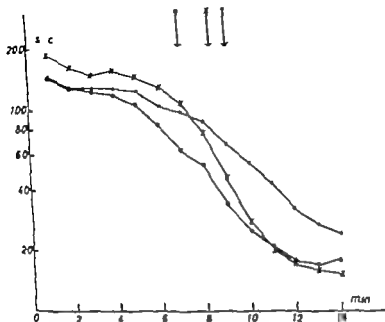


Fig 3 Thromboplastin activation curve of venous cord blood from 20 newborn infants whose mothers had not received vitamin K

x — x 15 normal adult women, two-stage TAT  
 ● — ● 20 normal newborn infants two-stage TAT  
 ○ — ○ 20 normal newborn infants, three-stage TAT

The fibrinolytic activity was determined in untreated plasma as well as in the ruglobulin fraction after iso-electric precipitation at pH 5.9 (Astrup and Rasmussen 1958)

(11) Haematocrit measured in per cent, determined in Wintrobe haematocrit tubes (Wintrobe 1956)

(12) Platelet counts after brief centrifugation of citrate-stabilized blood at 1500 p.m. The counting was done in Thoma chamber with depth of 0.05 mm, using phase contrast microscope

### Results

The results of the coagulation tests are listed in Table I. The curves representing TAT and the recalcification time are plotted in Figs. 1, 2, 3, 4 and 5.

Table 1 Results of Coagulation Analyses

Tests see Methods	Normal Women (not pregnant)		Normal Women (immediately after delivery)		Normal Newborn Infants		Prophylactic Administration of Vitamin K Normal Women (immediately after delivery)		Normal Newborn Infants	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets (thousands per cu mm.)	352 n = 25	223-498 ± 77	207 n = 20	67-351 ± 76	323 n = 20	66-571 ± 147	221 n = 20	142-383 ± 70	527 n = 20	224-863 ± 207
Prothrombin (per cent)	104 n = 25	85-145 ± 16	110 n = 20	80-190 ± 31	115 n = 20	62-170 ± 34	122 n = 20	75-210 ± 37	122 n = 20	82-160 ± 30
Prothrombin time (seconds)	18 n = 25	15-21 ± 1.2	17 n = 20	16-20 ± 0.9	19 n = 20	17-22 ± 1.5	17 n = 20	14-20 ± 1.4	19 n = 20	18-21 ± 0.9
PT (per cent)	103 n = 25	76-120 ± 13	206 n = 20	120-360 ± 68	72 n = 20	30-115 ± 25	227 n = 20	165-340 ± 51	90 n = 20	62-150 ± 21
Partial thromboplastin time (seconds)	81 n = 25	69-100 ± 5.9	73 n = 20	66-79 ± 3.6	88 n = 20	78-125 ± 11	75 n = 20	65-81 ± 4.0	92 n = 20	79-135 ± 12
Thrombin time (seconds)	9 n = 25	7-12 ± 1.0	8 n = 20	5-10 ± 1.1	13 n = 20	10-15 ± 1.5	8 n = 20	5-10 ± 1.6	14 n = 20	9-19 ± 2.5
Fibrinogen (mg. per 100 ml)	271 n = 25	165-360 ± 45	508 n = 20	320-965 ± 154	242 n = 20	150-340 ± 50	502 n = 20	380-690 ± 88	239 n = 20	130-345 ± 51
Haematocrit (per cent)	40 n = 25	34-46 ± 3.1	42 n = 20	36-48 ± 3.2	55 n = 20	48-63 ± 4.9	41 n = 20	34-46 ± 3.2	56 n = 20	46-62 ± 3.6

 $\bar{x}$  = arithmetic average n = number of estimationsrange = range of individual results ( $x$ ) est. SD = estimated standard deviation =  $\pm \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$ 

Clotting times shorter than 60 seconds in the partial thromboplastin time test are not included in the calculations. They were observed in one sample †



Table I. Results of Coagulation Analyses

Tests see Methods	Normal Women (not pregnant)		Normal Women (immediately after delivery)		Normal Newborn Infants		Prophylactic Administration of Vitamin K Normal Women (immediately after delivery)		Normal Newborn Infants	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets (thousands per cu mm)	352 n = 25	223 - 498 ± 77	207 n = 20	67 - 351 ± 76	323 n = 20	86 - 571 ± 147	221 n = 20	142 - 383 ± 70	527 n = 20	224 - 863 ± 207
Prothrombin (per cent)	104 n = 25	85 - 145 ± 16	110 n = 20	80 - 190 ± 31	115 n = 20	62 - 170 ± 34	122 n = 20	75 - 210 ± 37	122 n = 20	82 - 160 ± 30
Prothrombin time (seconds)	18 n = 25	15 - 21 ± 12	17 n = 20	16 - 20 ± 0.9	19 n = 20	17 - 22 ± 1.5	17 n = 20	14 - 20 ± 1.4	19 n = 20	18 - 21 ± 0.9
PT (per cent)	103 n = 25	76 - 120 ± 13	206 n = 20	120 - 380 ± 68	72 n = 20	30 - 115 ± 25	227 n = 20	105 - 340 ± 51	90 n = 20	62 - 150 ± 21
Partial thromboplastin time (seconds)	81 n = 25	69 - 100 ± 5.9	73 n = 20	66 - 79 ± 3.6	88 n = 20	78 - 125 ± 11	75 n = 19	65 - 81 ± 4.0	92 n = 20	79 - 135 ± 12
Thrombin time (seconds)	9 n = 25	7 - 12 ± 1.0	8 n = 20	5 - 10 ± 1.1	43 n = 20	10 - 15 ± 1.5	8 n = 20	5 - 10 ± 1.6	14 n = 20	9 - 19 ± 2.5
Fibrinogen (mg. per 100 ml)	271 n = 25	185 - 360 ± 45	508 n = 20	320 - 985 ± 154	242 n = 20	150 - 340 ± 50	502 n = 20	380 - 690 ± 88	230 n = 20	130 - 345 ± 51
Hematocrit (per cent)	40 n = 25	34 - 46 ± 3.1	42 n = 20	36 - 48 ± 3.2	55 n = 20	48 - 63 ± 4.4	41 n = 20	34 - 46 ± 3.2	56 n = 20	46 - 62 ± 3.6

 $\bar{x}$  = arithmetic average n = number f estimationsrange = range of individual results ( $\bar{x}$ ) est. SD = estimated standard deviation =  $\pm \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$ 

Clotting times shorter than 60 seconds in the partial thromboplastin time test are not included in the calculations. They were observed in one sample.

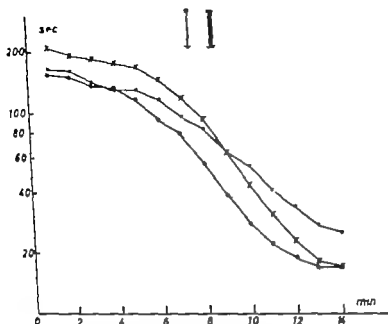


Fig 5 Thrombopilestin activation curve of venous cord blood from 20 newborn infants whose mothers had received vitamin K.

- 20 normal adult women, two-stage TAT
- 20 normal newborn infants, two-stage TAT
- 20 normal newborn infants, three-stage TAT

PP indicates distinctly that prothrombin-proconvertin, and possibly also the included factor X content, were significantly increased ( $p < 0.001$ )

The fibrinogen content was significantly increased ( $p < 0.001$ ) and the thrombin time shortened ( $p < 0.001$ )

The haematocrit was unchanged.

B Cord blood The platelet count was unchanged.

Two-stage TAT showed a significant shortening of  $t_1$  ( $p < 0.001$ ) but no change of  $t$  and  $T_{max}$ , while a significant prolongation of  $t_{max}$  ( $p < 0.001$ ) and of the recalcification time ( $0.02 > p > 0.01$ )

Three-stage TAT showed the same changes as for the mothers, i.e. a significant shortening of  $t_1$ ,  $t_r$  and of the recalcification time ( $p < 0.001$ ) but no change of  $T_{max}$  or  $t_{max}$ .

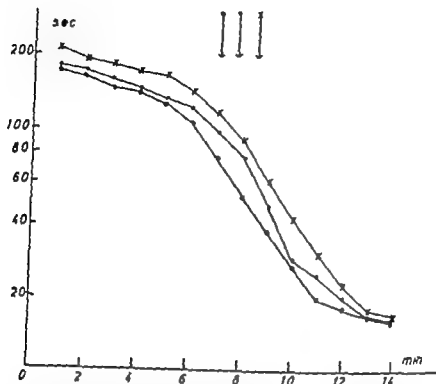


Fig. 4 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after delivery Prophylactic administration of vitamin K.  
 x—x 20 normal adult women two-stage TAT  
 ●—● 20 normal women immediately after delivery two-stage TAT  
 ○—○ 20 normal women immediately after delivery three stage TAT

Comparison of the values found in normal non-pregnant women (group 1) and in normal women immediately post partum as well as in their newborn infants (group 2) showed the following

A. *Peripheral maternal blood* A significant reduction of platelet count ( $p < 0.001$ )

Two-stage TAT revealed a significant shortening of  $t_1$  ( $0.01 > p > 0.001$ ) and of the recalcification time ( $0.05 > p > 0.02$ ) and a tendency to shortening of  $t_2$ . On the other hand,  $T_{max}$  and  $t_{max}$  were unchanged.

Three-stage TAT showed a significant shortening of  $t_1$ ,  $t_2$  and of the recalcification time ( $p < 0.001$ ) but no change in  $T_{max}$  or  $t_{max}$ .

A significant shortening of PTT ( $p < 0.001$ ) and of the Quick time ( $0.05 > p > 0.02$ ) while factor V remained unchanged



Comparison of group 2 and 3 shows that vitamin K has no influence upon the clotting and fibrinolysis factors in the peripheral maternal blood.

In cord blood the platelet count was significantly higher in the group where the mothers received vitamin K ( $0.01 > p > 0.001$ ).

The PP in the group where the mothers did not receive vitamin K was significantly lower than in the group treated prophylactically with this vitamin ( $0.05 > p > 0.02$ ).

Vitamin K exerted no influence upon fibrinolysis.

### Discussion

#### Coagulation

In the peripheral maternal blood there was a reduction of platelet count, but otherwise an increased content of the first-phase coagulation factors as expressed by the two-stage TAT. This is also compatible with a shortened PTT which is, essentially, a screening test for the entire coagulation mechanism, except for factor VII. On the basis of these two tests it is not possible to decide whether the increase is in factor VIII or factor IX—or perhaps both.

The three-stage TAT showed a significant shortening of  $t_1$ ,  $t_2$  as well as of the recalcification time. This must be taken to indicate that after normal delivery the peripheral maternal blood contains clot-promoting components.

In premature separation of the placenta and at times also in eclampsia Schneider (1951) demonstrated that thromboplastin, presumably of tissue origin, passes from the retroplacental or intradecidual haematoma to the maternal circulation leading to intravascular coagulation. It might be imagined that the same occurs in association with normal delivery only to a much

Being suspected that the increased platelet count of newborn children, whose mothers had received prophylactic vitamin K, might be due to systematic error re-investigations have been made on another group of newborn children. These investigations proved that the platelet count in the umbilical cord blood was independent of the prophylactic vitamin K received by the mothers.

A significant prolongation of PTT ( $0.02 > p > 0.01$ ) and of the Quick time ( $0.05 > p > 0.02$ ) while factor V was unchanged, and PP significantly reduced ( $p < 0.001$ )

The fibrinogen content showed a tendency to be reduced, while the thrombin time was significantly prolonged ( $p < 0.001$ )

The haematocrit was significantly elevated ( $p < 0.001$ )

The result of the *fibrinolysis studies* is listed in Table II. Comparison of group 1 with group 2 showed the following:

A. *Peripheral maternal blood* Plasminogen content was found to be significantly elevated ( $p < 0.001$ ) Fibrinolytic activity could not be demonstrated, either on standard fibrin plates or on heated plates. This indicates the absence of activator activity as well as of circulating plasmin. In the euglobulin fraction on the other hand, there was a significant increase in fibrinolytic activity on standard fibrin plates as well as on heated plates ( $p < 0.001$ )

This difference between the fibrinolytic activity in precipitated and non-precipitated plasma which is significant ( $p < 0.001$ ) may presumably be taken to indicate the presence of inhibitors in the plasma.

B *Cord blood* Plasminogen content was significantly reduced ( $p < 0.001$ ) Increased fibrinolytic activity was found in non-precipitated plasma on standard as well as on heated fibrin plates ( $p < 0.001$ ) indicating the presence of activator-activity as well as of circulating plasmin. In the euglobulin fraction there was also a significant increase ( $p < 0.001$ ) in fibrinolytic activity on standard as well as on heated fibrin plates. This finding too must be taken to indicate the presence of activator activity as well as of plasmin.

When considering the difference in fibrinolytic activity between non-precipitated and precipitated plasma, the activity is significantly higher in the euglobulin fraction ( $p < 0.001$ ) As stated above this may indicate the presence of inhibitors in the plasma.

#### *Influence of Vitamin K upon the Clotting and Fibrinolysis Factors*

Tables I and II give the results of the coagulation and fibrinolysis tests in cases where the mothers received prophylactic vitamin K (group 3)

As far as the 2nd and 3rd phases of coagulation are concerned, the results agree with those of previous authors.

The prolonged thrombin time in cord blood, combined with the modest reduction of fibrinogen, must be taken to indicate the presence of antithrombin. This also accords with the prolonged  $t_{max}$  in the two-stage TAT. To investigate this aspect in more detail, the following investigations were carried out after the completion of the main study.

In 10 samples of cord blood the thrombin time was found to average 12.3 sec. By replacing veronal buffer by imidazole blue, it was not possible to normalize the prolonged thrombin time, the mean value for the 10 tests being 11.1 sec. Thus, it may be established that the antithrombin present is not of heparin nature, but possibly it may have been formed as a consequence of the modest intravascular coagulation or of the presence of fibrinolytic split products.

The result of the haematocrit determinations agrees with previous findings.

### Fibrinolysis

It is apparent from the results found in elucidating fibrinolysis that an essential difference exists between the findings in mothers immediately post partum and in their newborn infants.

In the peripheral maternal blood the enzyme pre-stage plasminogen was significantly increased. In the euglobulin fraction there was a significant increase in activator-activity and in plasmin. Since this did not manifest itself in fibrinolytic activity in non-precipitated plasma, it may be assumed that the inhibitors have been sufficiently increased to balance with the fibrinolytic system at a higher level.

In cord blood the plasminogen content was significantly reduced. The reason may be either reduced production or increased consumption.

A reduced production is indicated by the experiments of Beller et al. (1966) who gave the mothers infusions of EACA immediately before delivery. Although this substance passed the placental barrier there was no increase in the plasminogen content in the newborns.

slighter extent. The failure to find the TAT curve so characteristic of the presence of tissue thromboplastin (Astrup and Ollendorff 1961) may be due to disappearance of the tissue thromboplastin immediately after the intravascular coagulation (Astrup and Albrechtsen 1968 personal communication)

Wessler (1955) has demonstrated that serum possesses a pronounced clot promoting activity "serum thrombotic accelerator". Thus it is possible that the demonstrated clot-promoting component is identical with Wessler's serum factor which may have been released by the intravascular coagulation resulting from the tissue thromboplastin.

For the 2nd phase of coagulation the results are in agreement with previous findings. The reason why it is difficult to measure minor fluctuations in the prothrombin content by the Quick method is that the Quick time is a screening test which determines factors II V VII and possibly also X. Indeed, it has been demonstrated that an elevated factor V content may exert a normalizing effect upon the Quick time so that real reductions in prothrombin do not manifest themselves (Owren 1950 Schultze and Schwick 1953 b and Ferguson and Patch 1956)

The results of the fibrinogen determinations, the 3rd phase of coagulation are also in accordance with the results of previous investigations and this also applies to the haematocrit determinations.

In cord blood  $t_1$  was shortened in the two-stage TAT while the recalcification time was prolonged. The result of the three-stage TAT which demonstrated distinctly the presence of clot promoting components with shortened  $t_1$ ,  $t_2$  and recalcification time indicates a reduced content of the 1st-phase clotting factors. Indeed, this is also compatible with a prolonged PTT

In view of previous findings it must be assumed that there is only a question of a reduction of factor IX.

Thus it is possible that clot-promoting components pass from the maternal to the infant's blood during delivery. This possibly explains why many investigators have found shortened clotting times for whole blood in spite of reduction of several coagulation factors.

6. Fibrinogen was greatly increased in the mothers while in the infants there was a tendency to reduction.
7. No fibrinolytic activity was detected in the plasma of the mothers, but a highly increased fibrinolytic activity with circulating plasmin, in the newborn.
8. The plasminogen content was considerably elevated in the mothers, but considerably reduced in the infants.
9. The haematocrit was unchanged in the mothers, but elevated in the infants.
10. Vitamin K has no influence upon maternal coagulation. On the other hand, the group of newborn infants whose mothers had received vitamin K showed a higher prothrombin-proconvertin content than the group whose mothers had not received this prophylaxis.
11. Vitamin K has no influence upon the fibrinolytic components, either in the mothers or in the newborn infants.

## REFERENCES

- Alexander B, Meyers L, Kenny J, Goldstein R, Gunnick V and Graspsoon L. *New Engl J Med* 254 358, 1956
- Alpersug, N, Fletcher A P and Sherry S. *J clin Invest* 38 1086, 1959
- Ambros C M, Weinstaub D H, Daughy D, Dowd, J E, Pickens J W, Neuwander K R and Ambros J L. *Pediatrics* 32 10, 1963
- Auer C J and Starup J. *Acta obstet gynec scand* 46 79 1957
- Astrup T and Møller S. *Arch Biochem* 40 346, 1952
- Astrup T and Rasmussen J. *Proc VII International Hematol Congr Rome*, 1958
- Astrup T and Øllendorff P. *J clin Lab Invest* 13 377 1961
- Barkhan P. *Brit J Haemat* 3 215 1957
- Beller F K. *Die Gerinnungsverhältnisse bei der Schwangeren und beim Neugeborenen*, J A Barth, Leipzig, 1957
- Beller F K, Goebelmann U, Douglas G W and Johnson A. *Obstet. and Gynec* 23 12, 1964
- Beller F K, Douglas, G W and Epstein, M. D. *Amer J Obstet Gynec* 66 577 1966
- Brünnel H W Z. *Geburtsh Gynäk* 135 40 1951
- Burglund G. *Acta paediat* 47 511 1958
- Foss R and Macfarlane R G. *Human Blood Coagulation and Its Disorders*, Blackwell Scientific Publ. Oxford, 1955
- Bukich G P, Rosenbaum W M and Sage E C., *J Amer med Ass* 116 1763 1941

As is apparent from the results in Table II there must, however also be a question of an increased consumption, as there was a brisk fibrinolytic activity with circulating plasmin.

The cause of this brisk fibrinolytic activity must be assumed to be the effect upon the infant during delivery as well as the reduction in the pH of the infant's blood immediately after birth (Engstrom and Kager 1964). However it is possible also that there may be a question of intravascular coagulation with secondary fibrinolysis.

### *Influence of Vitamin K upon the Coagulation and Fibrinolysis Factors*

The results showed that vitamin K administered to the mother before delivery has no influence upon the coagulation in the peripheral maternal blood immediately post partum. On the other hand, it was found in agreement with a number of previous studies that in the cord blood the PP was lower in newborn infants whose mothers had not received vitamin K than in the group where this prophylaxis had been used.

Vitamin K was not found to affect fibrinolysis either in the peripheral maternal blood or in the cord blood.

### SUMMARY

A study of coagulation and fibrinolysis components in normal women immediately post partum and in their newborn infants, including the influence of prophylactic vitamin K upon these components gave the following results:

- 1 The platelet count was reduced in the maternal blood while no definite changes were found in the newborn infants.
- 2 The two-stage TAT showed the factors in the first stage of coagulation to be increased in the mothers and reduced in the infants.
- 3 The three-stage TAT showed clot promoting factors in the blood of the mothers as well as of the infants.
- 4 Proaccelerin was unchanged in mothers as well as infants.
- 5 The prothrombin-proconvertin content was greatly elevated in the mothers, but reduced in the infants.

- Philips, L. L. and Schroeder V. *Pediatrics* 22 715, 1958
- Quick, A. J. *J. biol. chem.* 73, 109 1935
- Quick, A. J. Muraw L. G., Hussey C. V. and Burgess G. F., *Surg. Gynec. Obstet.* 55 671 1952
- Ramoff O. D. and Holland T. R., *Ann. N. Y. Acad. Sci.* 75 626, 1959
- Runge H. Hartert J. and Escher W. *Gynæcologia* 138 337 1954
- Semertzis, E. A. Cook C. D. and Rudolph A. J. *Acta paediat.* 49 727 1960
- Sanford, H. N. and Shwedginsky I. *Amer. J. Dis. Child.* 63 729 1942
- Schweder C. L., *J. Obstet. Gynec. Brit. Emp.* 58 538, 1951
- Schulze H. E. and Schwick, G. *Laboratoriums-Bilätter (Behring-Werke)* No. 3, 1953
- Schulze H. E. and Schwick, G. *Medizinische* 2 1354, 1953 b
- Schwenker A. W. Klotzsch B. and Roha, L. *Arch. Gynäk.* 191 8, 1958
- Shaper A. G. Macintosh, D. M. Evans C. M. and Kyobe J. *Lancet* 2 706, 1963
- Sjogvist P. and Albrechtsson O. K. *Acta obstet. gynec. scand.* 44 416, 1965
- Taylor F. M. *Pediatrics* 19 233, 1957
- Thorsderson O. *Undersøgelser over prothrombin hos sunde og syge* Universitetsforlaget, Århus, 1941
- Voss M. and Meyer W. *Ann. paediat.* 189 282, 1957
- Wagh, T. R. Marchant F. T. and Maughan, G. B. *Amer. J. med. Sci.* 198 64a, 1939
- Wessler S. J. *clin. Invest.* 34 647 1955
- Wintrobe M. M. *Clinical Hematology* Lea & Febiger Philadelphia, 1956

Received on April 1 1968

- Brakman P Amer J Obstet. Gynec. 94 14 1966
- Brakman P Albrechtsen O K. and Astrup T Brit. J Haemat. 12 74 1966
- Brakman P Albrechtsen O K. and Astrup T J Amer med. Ass. 199 68, 1967
- Cope I and Mitchell P Aust. N. Z. J Obstet. Gynaec. 4 117 1964
- Dyggve H V Undersøgelser over K vitaminets betydning for blodninger hos nyfødte Nyt Nordisk Forlag, København, 1952
- Dyggve H V Acta paediat. 47 251 1958
- Engström L and Lager L. Acta paediat. 53 329 1964
- Ferguson J H and Patch M J Proc. Soc. exp. Biol. 93 193 1956
- Fordecs J Németh L. and Elek E. Gynaecologia 154 29 1962
- Fresh J W Ferguson J H and Lewis J H J Obstet. Gynec. 7 117 1956
- Fresh J W Ferguson J H Stamey C Morgan F M and Lewis J H, Pediatrics 19 241 1957
- Gairdner D Marks J and Roscoe J D Arch. Dis. Childh. 27 214 1952
- Hessehtine H C Amer J Obstet. Gynec. 33 684 1937
- Kasper C. K. Houg, M S Aggeler P M. and Stone S. Obstet. and Gynec. 24 242 1964
- Kruger F Virchows Arch. path. Anat. 106 1 1886
- Künzer W and Strödel J Ann. paediat. 188 147 1957
- Künzer W., Gerstenkorn B. and Krüsselmann W Ann. paediat. 202 6, 1964
- Laricu M J Soulier J P and Minkowski A. Étud. néo-natal. 1 39 1952
- Larsen H., Svingninger i prothrombinaktiviteten hos nyfødte E. Munksgaards forlag, København 1952
- Lassen M Acta physiol. scand 27 371 1952
- Markarian M Githens J H Jackson J J Bennon, A. E Lindley A Rozenblut E. Martorell R. and Lubchenko L O Amer J Dis. Child. 113 312 1967
- McCready R. L. Callahan E. T and Grandin, D J Amer J Obstet. Gynec. 42 398 1941
- Mugrage E R. and Andresen M I Amer J Dis Child. 51 775 1936
- Nilsen P A Acta obstet. gynec. scand. 42 suppl. 2 1963
- Nilsson I M and Kullander S Acta obstet gynec scand. 46 273, 1967 a
- Nilsson I M and Kullander S Acta obstet gynec scand 46 286 1967 b
- Nye S W Graham J B and Brinkhous K M Amer J med. Sci. 243 279 1962
- Ollendorff P and Astrup T A sensitized thromboplastin activation test, In Torben Geill, On his 70th birthday 14.8.1966 P From Hansen, V Aa. Porxman C Petri and E. Snorrason, (eds) Danmarks Apotekerforening, København, 1966
- Owren, P A. 1950 cit. efter Larsen, H. Svingninger i prothrombinaktiviteten hos nyfødte E. Munksgaards forlag, København 1952
- Owren P A. and As K. Scand. J clin. Lab Invest. 3 201 1951
- Pechet L. and Alexander B. New Engl. J Med. 265 1093 1961



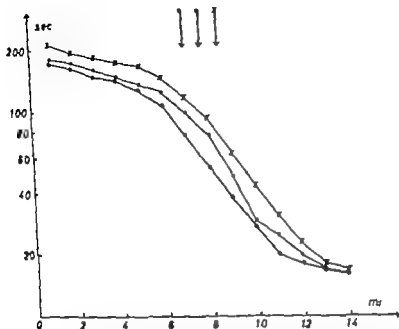


Fig 1 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after delivery Prophylactic administration of vitamin K.

- 20 normal adult women, two-stage TAT
- 20 normal women immediately after delivery two-stage TAT
- 20 normal women immediately after delivery three-stage TAT

In both categories the values are higher than in normal non-pregnant women. Newborn infants of diabetic mothers and premature infants of the same age (36–37 weeks) show no differences in fibrinolytic activity or plasminogen content, but if both groups are compared with normal full-term infants, the plasminogen content is found to be lower while the fibrinolytic activity is in the same range.

Andrus *et al* (1963) found the plasminogen content in five newborn of diabetic mothers to be in the same range as in normal full term or premature infants.

## COAGULATION AND FIBRINOLYSIS IN DIABETIC WOMEN IMMEDIATELY POST PARTUM AND IN THEIR NEWBORN INFANTS

Influence of Caesarean Section

BY

NIELS CHR. NIELSEN

In a previous study the author demonstrated various changes in coagulation and fibrinolysis in normal women immediately post partum and in their infants (Nielsen 1969). This confirmed in several respects the findings of others.

The object of the present study was to elucidate coagulation and fibrinolysis in diabetic women and their infants immediately post partum.

Since empirically diabetic women are not infrequently delivered by Caesarean section the study also included an investigation into the possible influence of this procedure upon the coagulation and fibrinolysis parameters.

### *Previous Investigations*

To the author's knowledge there have been no reports on coagulation in pregnant or parturient diabetics or their infants.

Studies on the haematocrit value in the cord blood of infants born to diabetic women have given conflicting results. Nicolopoulos and Smith (1961) found normal but Probst (1967) elevated values.

Plasminogen and fibrinolytic activity in the serum are identical in normal and diabetic parturient women (Samartzis et al 1960).

Table 1. Results of Coagulation and Fibrinolysis Studies in Women Immediately Post Partum

Tests see Methods	Normal Women		Women with Diabetes Mellitus			
			Vaginal Delivery		Caesarean Section	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets (thousands per cu mm)	221 -20	142-383 ±70	223 n=10	161-350 ±57	213 n=10	154-290 ±41
Prothrombin (per cent)	122 -20	75-210 ±37	120 n=10	77-185 ±31	110 n=10	69-200 ±38
Prothrombin time (seconds)	17 n=20	14-20 ±1.4	17 n=10	16-20 ±1.2	18 n=10	15-20 ±1.4
PT (per cent)	227 20	165-340 ±51	186 n=10	140-255 ±37	160 n=10	130-210 ±30
Partial thromboplastin time (seconds)	75 † 19	65-81 ±4.0	75 n=10	70-81 ±4.2	76 n=10	68-84 ±4.7
Thrombin time (seconds)	8 20	5-10 ±1.6	7 n=10	4-8 ±1.4	8 n=10	5-11 ±2.6
Fibrinogen (mg per 100 ml)	502 20	380-690 ±88	564 n=10	435-690 ±86	490 n=10	380-775 ±116
Fibratocrit (per cent)	41 20	34-46 ±3.2	47 n=10	41-50 ±3.6	42 n=10	35-46 ±3.8
Standard fibrin plates	0	0	0	0	0	0
Untreated plasmas (sq mm)	20	±0	n=10	±0	n=10	±0
Standard fibrin plates	37	11-75	27	4-49	15	0-40
Euglobulin (sq mm)	n=20	±17	n=10	±16	n=9	±15
Heated fibrin plates	0	0	0	0	0	0
Untreated plasmas (sq mm)	20	±0	n=10	±0	n=10	±0
Heated fibrin plates	25	9-78	28	16-38	17	2-36
Euglobulin (sq mm)	20	±16	10	±8.9	9	±11
Plasminogen (μg Co-Tyro- sine per ml)	168 20	126-208 ±21	176 10	144-208 ±18	163 n=10	121-222 ±32

arithmetical average      number of estimations

range of individual results ( )      est. SD      estimated standard deviation

$$\frac{\sum (x - \bar{x})^2}{n}$$

(Clotting times shorter than 60 seconds in the partial thromboplastin time test are not included in the calculations. They were observed in one sample.)

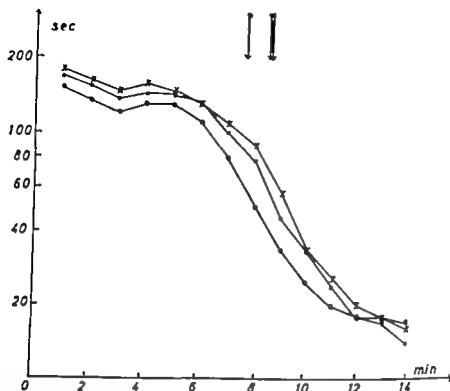


Fig. 2. Thromboplastin activation curve of peripheral blood from 10 women with diabetes mellitus immediately after delivery Caesarean section was not performed.

×—× 9 normal adult women, two-stage TAT

●—● 10 women with diabetes mellitus immediately after delivery two-stage TAT

○—○ 10 women with diabetes mellitus immediately after delivery three-stage TAT

### *Present Investigations*

#### *Material and Methods*

The analyses were carried out on blood samples from the following two groups

*Group 1* Ten women with diabetes mellitus whose samples were drawn within the first half hour after delivery. All had received prophylactic vitamin K in the form of menadione tablets 20 mg daily for about 8 days.

The average duration of diabetes was 5 years (4 months to 12

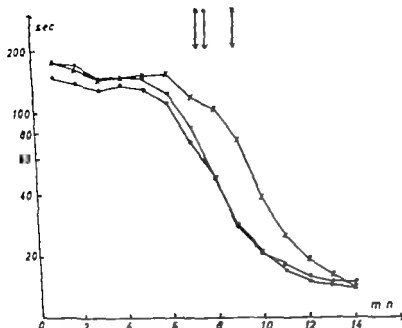


Fig. 3 Thromboplastin activation curve of peripheral blood from 10 women with diabetes mellitus immediately after delivery. Caesarean section was performed.

10 normal adult women, two-stage TAT

● 10 women with diabetes mellitus immediately after delivery two-stage TAT

○ 10 women with diabetes mellitus immediately after delivery three-stage TAT

years) No patient exhibited signs of nephropathy. All were well-controlled on insulin, and none had ketoacidosis.

Labour was induced about 3 weeks before term by intramuscular injections of Oxytocin followed by rupture of the membranes and an intravenous Oxytocin infusion if necessary. All deliveries were by the vaginal route.

Blood samples were also drawn from these women's newborn infants within the first 20 minutes after birth. The average birth weight was 3660 g (2700–4700 g). One infant died at 2 days of age of intracranial haemorrhage; the others exhibited no complications during their stay in hospital.

Table II *Results of Coagulation and Fibrinolysis Studies in Newborn Infants*

Tests see Methods	Normal Newborn		Newborn Infants of Diabetic Mothers			
	Infants		Vaginal Delivery		Caesarean Section	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets (thousands per cu.mm.)	577 n=20	44-803 $\pm 207$	253 n=10	176-425 $\pm 93$	786 n=10	57-663 $\pm 142$
Proaccelerin (per cent)	12 n=20	8-160 $\pm 30$	63 n=10	20-150 $\pm 35$	75 n=10	2-113 $\pm 30$
Prothrombin time (seconds)	19 n=20	18-21 $\pm 0.9$	2 n=10	19-29 $\pm 9$	1 n=10	17-7 $\pm 2.4$
PP (per cent)	90 n=70	62-150 $\pm 21$	57 n=10	31-100 $\pm 21$	46 n=10	11-50 $\pm 23$
Partial thromboplastin time (seconds)	97 n=70	79-135 $\pm 1$	99 n=10	81-166 $\pm 18$	109 n=10	83-171 $\pm 27$
Thrombin time (seconds)	14 n=20	9-19 $\pm 2.5$	16 n=10	11-31 $\pm 5.8$	17 n=10	9-31 $\pm 6.1$
Fibrinogen (mg per 100 ml.)	239 n=20	130-345 $\pm 51$	704 n=10	90-75 $\pm 66$	184 n=10	90-270 $\pm 59$
Haematocrit (per cent)	56 n=20	46-62 $\pm 3.6$	66 n=10	59-71 $\pm 4.1$	61 n=10	55-66 $\pm 3.5$
Standard fibrin plates	75	76-245	50	0-144	5	0-59
Untreated plasma (sq.mm.)	n=20	$\pm 51$	n=10	$\pm 44$	n=10	$\pm 3$
Standard fibrin plates	283	107-576	253	-414	177	10-373
Euglobulins (sq.mm.)	n=20	$\pm 155$	n=10	$\pm 137$	n=10	$\pm 125$
Heated fibrin plates	25	4-49	13	0-56	6	0-25
Untreated plasma (sq.mm.)	n=20	$\pm 10$	n=10	$\pm 1$	n=10	$\pm 8.8$
Heated fibrin plates	38	25-54	8	4-49	4	9-36
Euglobulins (sq.mm.)	n=70	$\pm 8.2$	n=10	$\pm 14$	n=10	$\pm 9.1$
Plasminogen (g Co-Tyro- sine per ml.)	61 n=20	35-104 $\pm 19$	53 n=10	5-81 $\pm 26$	4 n=10	16-66 $\pm 16$

See footnote to Table I.

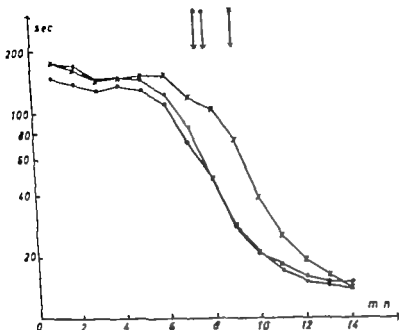


Fig 3 Thromboplastin activation curve of peripheral blood from 10 women with diabetes mellitus immediately after delivery Caesarean section was performed.

10 normal adult women, two-stage TAT

● 10 women with diabetic mellitus immediately after delivery two-stage TAT

● 10 women with diabetes mellitus immediately after delivery three-stage TAT

years) No patient exhibited signs of nephropathy. All were well-controlled on insulin, and none had ketoacidosis.

Labour was induced about 3 weeks before term by intramuscular injections of Oxytocin, followed by rupture of the membranes and an intravenous Oxytocin infusion if necessary. All deliveries were by the vaginal route.

Blood samples were also drawn from these women's newborn infants within the first 20 minutes after birth. The average birth weight was 3660 g (2700–4700 g). One infant died at 2 days of age of intracranial haemorrhage; the others exhibited no complications during their stay in hospital.

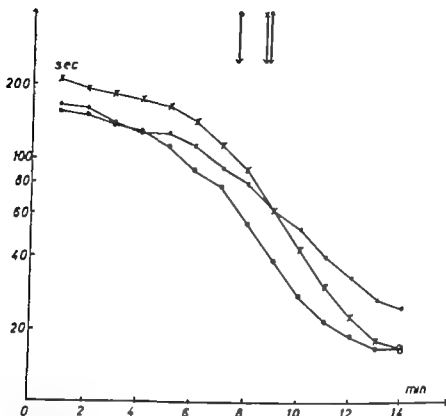


Fig. 4. Thromboplastin activation curve of venous cord blood from 20 newborn infants whose mothers had received vitamin K.  
 ×—× 20 normal adult women, two-stage TAT  
 ●—● 20 normal newborn infants two-stage TAT  
 ○—○ 20 normal newborn infants three-stage TAT

**Group 2** Ten diabetic women and their infants. Vitamin K prophylaxis and blood sampling was as described under group 1 (However two patients did not receive vitamin K, as delivery occurred before the prophylaxis had been started. The analytical results in these two cases did not differ from those in the others)

The average duration of diabetes was 8 years (6 months to 14 years) No patient exhibited signs of nephropathy All were on insulin and well-controlled, without signs of ketoacidosis.

Labour was induced about 3 weeks before term except in the two cases where labour started spontaneously All these women were delivered by Caesarean section owing to threatened or mani-



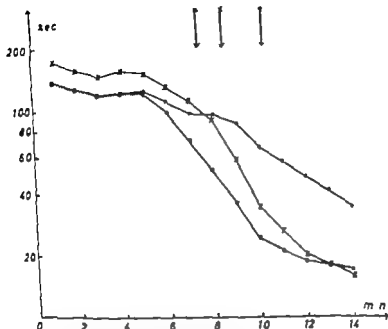


Fig 5 Thromboplastin activation curve of venous cord blood from 10 newborn infants of diabetic mothers. Caesarean section was not performed.

9 normal adult women, two-stage TAT

● 10 newborn infants of diabetic mothers, two-stage TAT

○ 10 newborn infants of diabetic mothers, three-stage TAT

test complications due to the diabetes. The infants' average birth weight was 3350 g (2300–4380 g). One infant died at 8 hours of age and autopsy showed total atelectasis of the lungs. The others showed no complications while in hospital.

Group 3 was a control group of 20 normal parturient women and their newborn infants. Vitamin K was administered and blood samples drawn in the same way as described for the two groups of diabetic women. This control series has been published previously (Nielsen 1969).

The technique of blood sampling and further treatment of the samples was as described in the previous paper. The determination of the recalcification time, thromboplastin activity test (TAT), two- and three-stage partial thromboplastin time (PTT), thrombus time, prothrombin time (Quick) factor

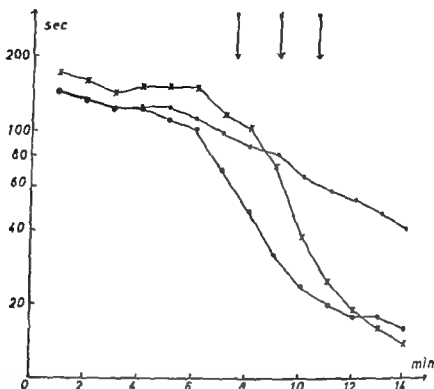


Fig. 6. Thromboplastin activation curve of venous cord blood from 10 newborn infants of diabetic mothers, Caesarean section was performed.  
 x—x 10 normal adult women, two-stage TAT  
 ●—● 10 newborn infants of diabetic mothers, two-stage TAT  
 ○—○ 10 newborn infants of diabetic mothers three-stage TAT

V prothrombin-proconvertin (PP) fibrinogen content, and determination of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on untreated fibrin plates (standard plates) and on heated fibrin plates, as well as plasminogen haematocrit, and platelet counts were also done as described in the previous paper (Nielsen 1969)

### Results

The results of the coagulation studies are given in Tables I and II and those of the TAT and recalcification times in Figs. 1 2, 3 4 5 and 6

On comparison between group 1 (diabetic mothers and their infants) and group 3 (normal mothers and their infants) the following observations were made

A. *Maternal peripheral blood.* Apart from the fact that the diabetic had a prolonged recalcification time in TAT two-stage ( $0.02 > p > 0.01$ ) a reduced PP content ( $0.05 > p > 0.02$ ) and elevated haematocrit ( $p < 0.001$ ) there were no significant differences.

B. *Cord blood.* In the infants of diabetic mothers the platelet count was significantly reduced ( $p < 0.001$ ) TAT two-stage showed a flatter course, with a significantly shortened  $t_1$  ( $p < 0.001$ ) unchanged  $t_2$  but prolonged  $t_{\max}$ . There was no difference in  $T_{\max}$  but a significantly prolonged recalcification time ( $0.05 > p > 0.02$ )

TAT three-stage showed, as in the mothers, significant activation, in the same range in the newborn infants of diabetic women as in normal newborns.

In newborn infants of diabetic mothers factor V was significantly reduced ( $0.01 > p > 0.001$ ) PP reduced ( $p < 0.001$ ) and the Quick time significantly prolonged ( $0.01 > p > 0.001$ )

Otherwise, there were no significant changes in the coagulation parameters. The haematocrit value was significantly higher in the diabetic group ( $p < 0.001$ )

The results of the *fibrinolysis studies* are also listed in Tables 1 and 2. Comparison between group 1 and group 3 shows

A. *Maternal peripheral blood.* No significant difference between the two groups.

B. *Cord blood.* Both groups showed marked fibrinolytic activity in unprecipitated and precipitated plasma, on standard as well as on heated fibrin plates. However the fibrinolytic activity was more pronounced in normal newborn infants, but this difference was significant only on heated fibrin plates ( $0.01 > p > 0.001$ ) in untreated plasma, and ( $0.02 > p > 0.01$ ) in the euglobulin fraction.

Plasminogen content was slightly but not significantly reduced in the diabetic group

On supplemental examination, confer *Asche* (1969) this result must be corrected in such way that the conclusion is that there is no difference in the platelet count for children borne by mothers with diabetes mellitus and for normal newborns.

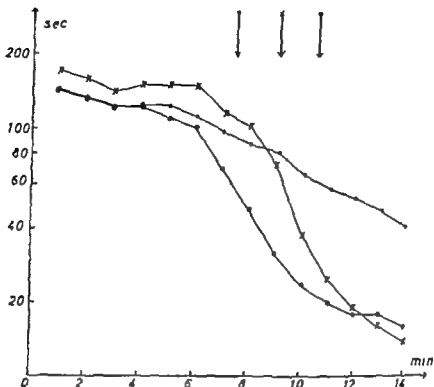


Fig. 6. Thromboplastin activation curve of venous cord blood from 10 newborn infants of diabetic mothers. Caesarean section was performed.  
 ×—× 10 normal adult women, two-stage TAT  
 ●—● 10 newborn infants of diabetic mothers, two-stage TAT  
 ○—○ 10 newborn infants of diabetic mothers three-stage TAT

V prothrombin-proconvertin (PP) fibrinogen content and determination of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on untreated fibrin plates (standard plates) and on heated fibrin plates, as well as plasminogen haematocrit, and platelet counts were also done as described in the previous paper (Nielsen 1969)

### Results

The results of the *coagulation studies* are given in Tables I and II and those of the TAT and recalcification times in Figs 1 2, 3 4 5 and 6

On comparison between group 1 (diabetic mothers and their infants) and group 3 (normal mothers and their infants) the following observations were made

and release of serum which, as demonstrated by Wessler (1955) possesses a coagulation-promoting activity (*cf* also Nielsen, 1969)

The reduced content of coagulation factors in the infants of the diabetic group may be due to an influence of maternal diabetes upon the foetal liver but it may also be a consequence merely of relative prematurity

The greatly elevated haematocrit, which makes the results even more striking, is possibly due to the greatly increased extramedullary haemopoiesis in these infants (Miller and Wilson 1943)

### *Fibrinolysis*

No difference was found in plasminogen content or fibrinolytic activity between the peripheral blood of diabetic mothers and normal mothers or between the cord blood of infants born to diabetic mothers compared with that of normal infants.

The highly increased fibrinolytic activity in the cord blood is presumably due to an influence upon the foetus during delivery as well as to the reduction in the pH of the infant's blood immediately after birth (Engström and Kager 1964) (*Cf* also Nielsen 1969)

The brisk fibrinolytic activity which gives rise to the production of an increased quantity of split products perhaps explains the prolonged thrombin time which does not return to normal upon addition of toluidine blue.

### *Coagulation and Fibrinolysis Factors Following Caesarean Section*

Caesarean section has no influence upon the coagulation or fibrinolysis factors as judged from samples of peripheral blood drawn from diabetic mothers immediately post partum. Also the cord blood of infants born to diabetic mothers delivered by Caesarean section showed no changes in coagulation factors. On the other hand, the fibrinolytic activity was slightly reduced, possibly as a result of a gentler birth.

### *Coagulation and Fibrinolysis Factors in Patients Delivered by Caesarean Section*

On comparison between group 1 (diabetic mothers delivered by the vaginal route and their infants) and group 2 (diabetic mothers delivered by Caesarean section and their infants) the following findings were made in respect to coagulation

A. *Maternal peripheral blood* Apart from a shorter recalcification time ( $0.02 > p > 0.01$ ) and  $t_{\max}$  ( $0.01 > p > 0.001$ ) in the TAT two stage in group 2 there were no significant differences in coagulation factors. The TAT three-stage indicated the same degree of activation in both groups.

B. *Cord blood* No significant differences between the two groups.

In respect to fibrinolysis the two groups showed

A. *Maternal peripheral blood* No significant differences between the two groups.

B. *Cord blood* No difference in plasminogen content. On the other hand, fibrinolytic activity was less pronounced in the infants of the group delivered by Caesarean section all the fibrinolysis values being lower than for vaginally delivered infants but the differences are not significant.

### *Discussion*

*Coagulation* Diabetic mothers proved to have a reduced prothrombin proconvertin level and a prolonged recalcification time as compared with normal mothers. These differences are significant. The explanation is presumably that the diabetic mothers were delivered about 3 weeks before term. Other coagulation factors showed no significant differences between the two groups.

The cord blood of infants born to diabetic mothers showed compared with that of normal newborns a reduction of all coagulation parameters except for fibrinogen. On the other hand the plasma level of coagulation activating components was as in normal infants. The explanation is presumably that at delivery tissue thromboplastin passes to the maternal circulation although only to a slight extent from a small retroplacental or intradecidual haematoma. This leads to intravascular coagulation

## COAGULATION AND FIBRINOLYSIS IN WOMEN DELIVERED BY ELECTIVE CAESAREAN SECTION AND IN THEIR NEWBORN INFANTS

BY

NIELS CHR. NIELSEN

In a previous communication the author reported a finding of coagulation-activating components in the maternal as well as cord blood after normal delivery (Nielsen 1969 a). As an explanation it was suggested that small quantities of tissue thromboplastin and serum might pass from a small retroplacental or intradecidual haematoma—similar to Schneider's (1951) findings for tissue thromboplastin in abruptio placentae and in eclampsia.

The object of the present study was to elucidate the coagulation and fibrinolysis factors in elective Caesarean section not preceded by labour or by damage to the placenta.

### *Previous Investigations*

To the author's knowledge there have been only a very few reports on coagulation and fibrinolysis in women delivered by elective Caesarean section and in their infants.

Masure and Schonne (1956) studying coagulation in 24 newborn infants after normal delivery found hypercoagulability expressed by thrombelastographic changes and a shortened recalcification time in more than half the infants. In five infants delivered by elective Caesarean section the thrombelastography as well as the recalcification time were normal. In coagulation of infants delivered by Caesarean section during labour showed the

## SUMMARY

A study of the coagulation and fibrinolysis factors in diabetic women immediately post partum and in their infants, including the influence of Caesarean section upon these factors, revealed the following differences from normal mothers and their newborn infants

## A. In the maternal blood

- 1 Prolonged recalcification time in TAT two-stage, a reduced PP and elevated haematocrit.
2. No change in fibrinolysis factors

## B In cord blood

- 1 A flatter course of TAT two-stage with a prolonged recalcification time and t max. Reduced factor V and PP and a prolonged Quick Time. Greatly elevated haematocrit.
2. Reduced fibrinolytic activity on heated fibrin plates.

C No influence of Caesarean section upon maternal coagulation or fibrinolysis factors. Among the newborn infants, on the other hand the infants of diabetic mothers delivered by Caesarean section showed less fibrinolytic activity

## REFERENCES

- Ambrus C M, Weisraub D H, Dunphy D, Dours J E, Pickren J W, Nitsenwiler A. R. and Ambrus J L. *Pediatrics* 32 10 1963  
 Engström L and Kage L. *Acta paediat.* 53 329 1964  
 Miller H C and Wilson H M. *J Pediat* 23 251 1943  
 Nicolopoulos D A. and Smith C A. *Pediatrics* 28 206, 1961  
 Nielsen N C. *Acta obstet. gynec. scand.* 48 371 1969  
 Probst 1967 cit after Pedersen J. *The Pregnant Diabetic and Her Newborn*, Munksgaard Copenhagen, 1967  
 Samartzis E. A, Cook C D and Rudolph A J. *Acta paediat.* 49 727 1960  
 Wessler S. *J clin. Invest.* 34 647 1955

Received on Sept. 1 1968



## COAGULATION AND FIBRINOLYSIS IN WOMEN DELIVERED BY ELECTIVE CAESAREAN SECTION AND IN THEIR NEWBORN INFANTS

BY

NIELS CHR. NIELSEN

In a previous communication the author reported a finding of coagulation-activating components in the maternal as well as cord blood after normal delivery (Nielsen 1969 a). As an explanation it was suggested that small quantities of tissue thromboplastin and serum might pass from a small retroplacental or intradecidual haematoma—similar to Schneider's (1951) findings for tissue thromboplastin in abruptio placentae and in eclampsia.

The object of the present study was to elucidate the coagulation and fibrinolysis factors in elective Caesarean section not preceded by labour or by damage to the placenta.

### *Previous Investigations*

To the author's knowledge there have been only a very few reports on coagulation and fibrinolysis in women delivered by elective Caesarean section and in their infants.

Masure and Schonne (1956) studying coagulation in 24 newborn infants after normal delivery found hypercoagulability expressed by thrombelastographic changes and a shortened recalcification time in more than half the infants. In five infants delivered by elective Caesarean section the thrombelastography as well as the recalcification time were normal. Investigation of infants delivered by Caesarean section during labour showed the

## SUMMARY

A study of the coagulation and fibrinolysis factors in diabetic women immediately post partum and in their infants, including the influence of Caesarean section upon these factors, revealed the following differences from normal mothers and their newborn infants

## A In the maternal blood

- 1 Prolonged recalcification time in TAT two-stage a reduced PP and elevated haematocrit.
2. No change in fibrinolysis factors.

## B In cord blood

- 1 A flatter course of TAT two-stage with a prolonged recalcification time and t max Reduced factor V and PP and a prolonged Quick Time Greatly elevated haematocrit.
2. Reduced fibrinolytic activity on heated fibrin plates.

C. No influence of Caesarean section upon maternal coagulation or fibrinolysis factors Among the newborn infants on the other hand the infants of diabetic mothers delivered by Caesarean section showed less fibrinolytic activity

## REFERENCES

- Ambros C M Weintraub D H Dunphy D Dours J E Pickren J W  
 Niswander K. R. and Ambros J L. *Pediatrics* 32 10 1963  
 Engström L. and Kager L. *Acta paediat* 53 329 1964  
 Miller H C and Wilson H M. *J Pediat* 23 251 1943  
 Nicolopoulos D. A. and Smith C. A. *Pediatrics* 28 206, 1961  
 Nielsen, N. C. *Acta obstet gynec. scand.* 48 371 1969  
 Probst 1967 cit. after Pedersen J *The Pregnant Diabetic and Her Newborn*,  
 Munksgaard Copenhagen, 1967  
 Samuels E. A. Cook C. D. and Rudolph A. J. *Acta paediat* 49 727  
 1960  
 Wessler S. *J clin Invest.* 34 647 1955

Received on Sept 1 1968

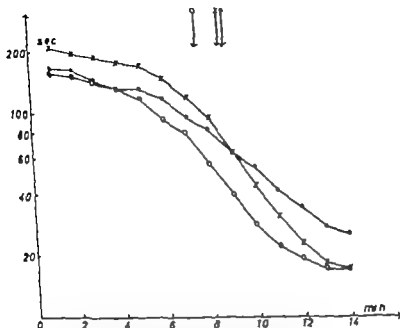


Fig 2 Thromboplastin activation curve of venous cord blood from 20 normal newborn infants immediately after normal vaginal delivery

- 20 normal adult women, two-stage TAT
- 20 normal newborn infants, two-stage TAT
- 20 normal newborn infants three-stage TAT

section at term by a transverse incision into the uterus. The blood samples were drawn within the first 20 minutes after removal of the placenta. The indication for the operation was usually previous Caesarean section or mechanical disproportion. In no case was it done because of pregnancy complications such as pre-eclampsia, abruptio placentae etc.

After premedication with atropine, the operation was carried out under general anaesthesia, induced by barbiturate, suxamethonium chloride and intubation, and maintained by nitrous oxide, cyclopropane and oxygen. During the procedure glucose was infused, but never blood prior to the removal of the blood samples.

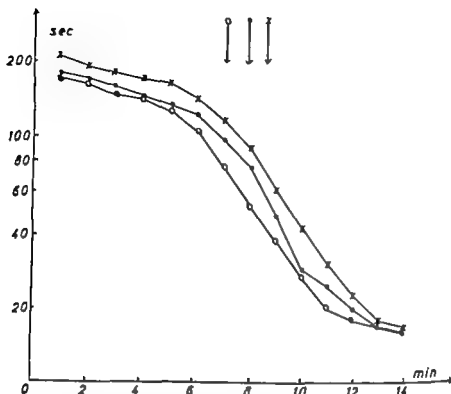


Fig 1 Thromboplastin activation curve of peripheral blood from  $\square$  normal women immediately after normal vaginal delivery  
 $\times-\times$  20 normal adult women two-stage TAT  
 $\bullet-\bullet$  20 normal women immediately after delivery two-stage TAT  
 $\bigcirc-\bigcirc$  20 normal women immediately after delivery three-stage TAT

same thrombelastographic changes as following normal vaginal delivery. It was concluded that the tendency to the hypercoagulability in newborn infants was due mainly to thromboplastic factors of placental origin, liberated in the foetal circulation during labour.

### *Present Investigations*

#### *Material and Methods*

The analyses were performed on blood samples from the following two groups.

*Group 1* Fifteen normal women delivered by elective Caesarean

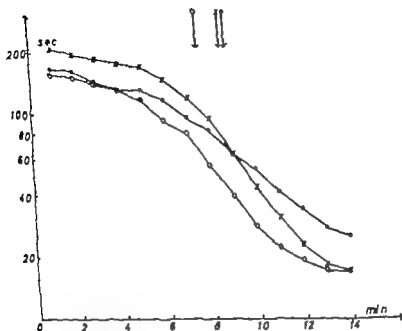


Fig. 2 Thromboplastin activation curve of venous cord blood from 20 normal newborn infants immediately after normal vaginal delivery

- 20 normal adult women, two-stage TAT
- 20 normal newborn infants, two-stage TAT
- 20 normal newborn infants, three-stage TAT

section at term by a transverse incision into the uterus. The blood samples were drawn within the first 20 minutes after removal of the placenta. The indication for the operation was usually previous Caesarean section or mechanical disproportion. In no case was it done because of pregnancy complications such as pre-eclampsia, abruptio placentae, etc.

After premedication with atropine the operation was carried out under general anaesthesia, induced by barbiturate, succinylcholine chloride, and intubation, and maintained by nitrous oxide cyclopropane and oxygen. During the procedure glucose was infused but never blood prior to the removal of the blood samples.

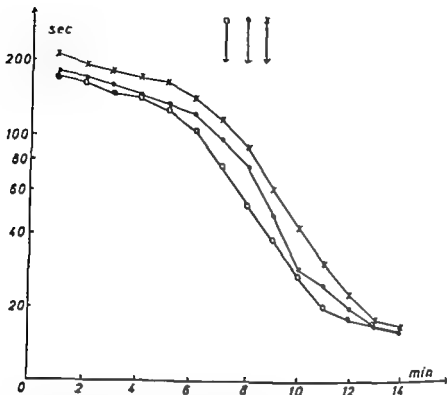


Fig. 1 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after normal vaginal delivery  
 x—x 20 normal adult women, two-stage TAT  
 ●—● 20 normal women immediately after delivery two-stage TAT  
 ○—○ 20 normal women immediately after delivery three-stage TAT

same thrombelastographic changes as following normal vaginal delivery. It was concluded that the tendency to the hypertoagulability in newborn infants was due mainly to thromboplastic factors of placental origin, liberated in the foetal circulation during labour.

### *Present Investigations*

#### *Material and Methods*

The analyses were performed on blood samples from the following two groups.

*Group 1* Fifteen normal women delivered by elective Caesarean

Table II. *Results of Coagulation and Fibrinolysis Studies in Normal Newborn Infants*

Tests, see Methods	Normal Newborn Infants			
	Vaginal Delivery		Elective Caesarian section	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets (thousands per cu mm.)	527 - 20	224-863 $\pm 207$	367 n=15	225-522 $\pm 84$
Prothrombin (per cent)	122 n=20	82-160 $\pm 30$	104 n=15	72-143 $\pm 20$
Prothrombin time (seconds)	19 n=20	18-21 $\pm 0.9$	20 n=15	18-23 $\pm 1.4$
PT (per cent)	90 n=20	62-150 $\pm 21$	81 n=15	62-98 $\pm 11$
Partial thromboplastin time (seconds)	92 - 20	79-135 $\pm 12$	98 - 15	83-106 $\pm 8.5$
Thrombin time (seconds)	14 n=20	9-19 $\pm 2.5$	14 - 15	9-23 $\pm 3.5$
Fibrinogen (mg per 100 ml)	238 - 20	130-345 $\pm 51$	216 - 15	150-315 $\pm 49$
Haematocrit (per cent)	56 n=20	46-62 $\pm 3.6$	53 - 15	46-60 $\pm 4.1$
Standard fibrin plates	75	26-245	32	0-140
Untreated plasma (sq mm.)	20	$\pm 51$	- 15	$\pm 32$
Standard fibrin plates	283	107-576	167	11-466
Euglobulin (sq mm.)	n=20	$\pm 151$	n=15	$\pm 129$
Heated fibrin plates	25	4-49	14	0-25
Untreated plasma (sq mm.)	- 20	$\pm 10$	n=15	$\pm 10$
Heated fibrin plates	38	25-54	23	0-40
Euglobulin (sq mm.)	20	$\pm 8.2$	n=15	$\pm 12$
Plasminogen (ug C <sub>4</sub> -Tyro- sine per ml)	61 20	35-104 $\pm 19$	63 n=15	34-110 $\pm 21$

See footnote to Table I

Table I. Results of Coagulation and Fibrinolysis Studies in Women Immediately Post Partum

Tests see Methods	Normal Women			
	Vaginal Delivery		Elective Caesarean section	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets (thousands per cu. mm.)	221 n=20	147-383 ±70	229 n=15	155-296 ±44
Proaccelerin (per cent)	1.2 n=20	75-210 ±37	103 n=15	71-130 ±18
Prothrombin time (seconds)	17 n=20	14-20 ±1.4	18 n=15	15-21 ±1.5
PP (per cent)	227 n=20	165-340 ±51	186 n=15	140-240 ±20
Partial thromboplastin time (seconds)	75 n=19	65-81 ±4.0	75 n=15	69-82 ±3.5
Thrombin time (seconds)	8 n=20	5-10 ±1.6	8 n=15	6-10 ±0.6
Fibrinogen (mg per 100 ml)	502 n=20	380-690 ±88	473 n=15	345-580 ±121
Haematocrit (per cent)	41 n=20	34-46 ±3.2	39 n=15	31-47 ±4.6
Standard fibrin plates	0	0	0	0
Untreated plasma (sq. mm.)	n=20	±0	n=15	±0
Standard fibrin plates	37 n=20	11-75 ±17	16 n=15	0-79 ±20
Euglobulins (sq. mm.)	0	0	0	0
Untreated plasma (sq. mm.)	n=20	±0	n=15	±0
Husted fibrin plates	25 n=20	9-78 ±16	17 n=15	0-51 ±14
Euglobulins (sq. mm.)	168 n=20	126-208 ±21	173 n=15	150-206 ±14
Plasminogen ( $\mu$ g Cu-Tyrosine per ml)				

$\bar{x}$  = arithmetic average n = number of estimations  
range = range of individual results (x) est. SD = estimated standard deviation

$$= \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Clotting times shorter than 60 seconds in the partial thromboplastin time test are not included in the calculations. They were observed in one sample†



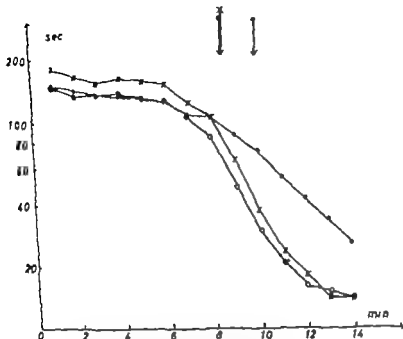


Fig 4 Thromboplastin activation curve of venous cord blood from 15 normal newborn infants delivered by elective Caesarean section  
 — 14 normal adult women two-stage TAT  
 ●—● 15 normal newborn infants delivered by elective Caesarean section two-stage TAT  
 ○—○ 15 normal newborn infants delivered by elective Caesarean section, three-stage TAT

**Group 2** This was a control group of 20 normal parturient women and their newborn infants. Vitamin K was administered and blood samples drawn just as in group 1. This control group has been described previously (Nielsen 1969 a).

The technique of the blood sampling and the further study of the samples were as described previously. The determination of recalcification time, the procedures of the thromboplastin activation test (TAT) two- and three-stage partial thromboplastin time (PTT) thrombin time prothrombin time (Quick) factor V prothrombin-proconvertin (PP) fibrinogen content, and the determination of fibrinolytic activity in the plasma and in iso-electrically precipitated plasmas on untreated fibrin plates (standard plates) and on

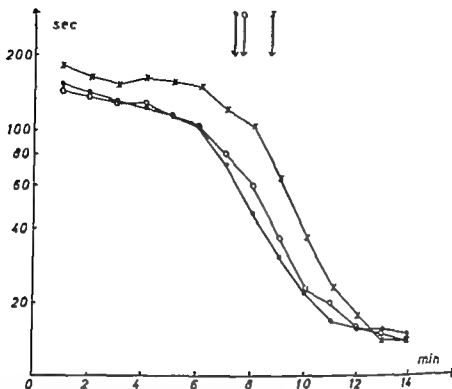


Fig. 3. Thromboplastin activation curve of peripheral blood from 15 normal women immediately after elective Caesarean section was performed.

×—× 14 normal adult women two-stage TAT

●—● 15 normal women immediately after elective Caesarean section, two-stage TAT

○—○ 15 normal women immediately after elective Caesarean section, three-stage TAT

No patient had been in labour prior to the delivery and in all cases the foetus was delivered without any damage to the placenta.

All the women (except for two whose analyses did not differ from the others) had received prophylactic vitamin K, in the form of menadione tablets, 20 mg daily prior to the operation.

Blood samples were also drawn from these women's newborn infants within the first 10 minutes after birth. All the infants were full term and none exhibited any complications while in hospital.

- Wiersma, W. C. *Acta obstet. gynec. scand.* 48 371 1969 a
- Wiersma, W. C. *Acta obstet. gynec. scand.* 48 392, 1969 b
- Haender, C. L. J. *Obstet. Gynec. Brit. Emp.* 58 538 1951
- Anderson, J. L., McGovern, J. J., Bunker, J. P. and Goldstein, R. *Anesthesiology* 23 92, 1962

ceived on Nov. 28, 1968

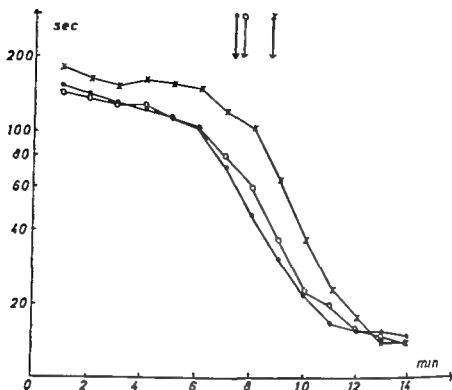


Fig. 3. Thromboplastin activation curve of peripheral blood from 15 normal women immediately after elective Caesarean section was performed.

×—× 14 normal adult women two-stage TAT

●—● 15 normal women immediately after elective Caesarean section, two-stage TAT

○—○ 15 normal women immediately after elective Caesarean section, three-stage TAT

No patient had been in labour prior to the delivery and in all cases the foetus was delivered without any damage to the placenta.

All the women (except for two whose analyses did not differ from the others) had received prophylactic vitamin K<sub>1</sub> in the form of menadione tablets 20 mg daily prior to the operation.

Blood samples were also drawn from these women's newborn infants within the first 10 minutes after birth. All the infants were full term and none exhibited any complications while in hospital.

The results of fibrinolysis studies are also listed in Tables 1 and 2. Comparison of group 1 and 2 shows

A. *Maternal peripheral blood* In the Caesarean section group the fibrinolytic activity in the euglobulin fraction on the standard plates was less than that following vaginal delivery ( $0.01 > p > 0.001$ ) but otherwise there were no significant differences between the two groups.

B. *Cord blood*. There was no difference in plasminogen content. On the other hand, fibrinolytic activity measured on the fibrin plates in the Caesarean section group was less marked, all the determinations of fibrinolysis being significantly lower than for the infants delivered by the vaginal route. The  $p$  values are thus, for unprecipitated plasma on standard plates ( $0.05 > p > 0.02$ ) and on heated plates ( $0.01 > p > 0.001$ ) and for the euglobulin fraction on standard plates ( $0.05 > p > 0.02$ ) and on heated plates ( $p < 0.001$ ).

### Discussion

In a previous paper (Nielsen 1969 b) it was demonstrated that *Caesarean section during labour* has no influence upon the coagulation factors in the maternal or in the cord blood. The fibrinolytic activity is also unchanged in the maternal blood following Caesarean section, while it is reduced in the newborn infants, presumably due to a more gentle manner of birth. In the maternal as well as in the cord blood there were clot promoting components of the same magnitude as following normal vaginal delivery.

In the present study it was demonstrated, by the TAT three-stage, that presumably less marked activation of the maternal plasma occurs after *elective Caesarean section* than after vaginal delivery. In the plasma of the newborn infants the TAT three-stage clearly demonstrated that no activation of the coagulation process takes place after delivery by elective Caesarean section, unlike the findings following vaginal delivery. These results support the theory that during normal delivery there will be activation of the maternal as well as of the infants' blood. As mentioned above, this may be due to the passage of tissue thromboplastin and serum to the maternal and foetal circulation during delivery.

heated fibrin plates as well as plasminogen, haematocrit, and platelet counts were also done as described previously (Nielsen 1969 a).

### Results

The results of the *coagulation studies* are given in Tables I and II, and those of the TAT and recalcification times in Figs. 1, 2, 3, and 4.

Comparison of group 1 (mothers delivered by elective Caesarean section and their infants) and group 2 (normal mothers and their infants) showed

*A. Maternal peripheral blood* The TAT two-stage showed no major differences between the two groups. However  $t_1$  was significantly shortened in the Caesarean section group ( $0.01 > p > 0.001$ ) while no significant changes for  $t_2$ ,  $t_{\max}$ , or for the recalcification times were demonstrated. In the TAT three-stage there are as described in a previous paper clot-promoting components in the maternal plasma following normal delivery (Nielsen 1969 a). In the Caesarean section group the three-stage curve is situated between the 2 two-stage curves except during the first minutes. The recalcification time for the three-stage curve in the Caesarean group is also somewhat longer than the recalcification time for the three-stage curve representing normal mothers.

No other significant differences were found, apart from a reduction in PP ( $0.01 > p > 0.001$ ) in the Caesarean section group.

*B. Cord blood* The recalcification time for infants delivered by Caesarean section was significantly prolonged in the TAT two-stage ( $0.05 > p > 0.02$ ) and the curve was a little flatter. The TAT three-stage showed practically no clot-promoting activity in the plasma of infants delivered by elective Caesarean section, unlike infants delivered by the vaginal route in whom the activity is in the same range as in the maternal blood.

Incidentally there were no changes in coagulation factors in infants delivered by elective Caesarean section apart from a significant reduction in platelet count ( $0.01 > p > 0.001$ ).

On supplemental examination, confer Nielsen (1969 a) this result must be corrected in such a way that the conclusion is that there is no difference of the platelet count for children delivered by elective Caesarean section and for infants delivered by the vaginal route.

- Nielens, N. C., *Acta obstet. gynec. scand.* 48: 371, 1969 a  
Nielens, N. C., *Acta obstet. gynec. scand.* 48: 392, 1969 b  
Schwaidler C. L. *J Obstet. Gynec. Brit. Emp.* 58: 538, 1951  
Vanderveen J. L., McGovern J. J., Bunker J. P. and Goldstein R. *Anesthesiology* 23: 92, 1962

Received on Nov. 28, 1968

Incidentally elective Caesarean section does not appear to cause major changes in maternal coagulation factors. The finding that the values for PP (186 per cent) was lower than following vaginal delivery (PP 227 per cent) does not explain, but presumably suggests inaccuracy of the method, the determination being based upon dilution curves plotted on logarithmic paper where the uncertainty is most marked in the high concentrations.

The flatter course of the TAT two-stage and the prolonged recalcification time in cord blood following elective Caesarean section accord with the fact that no activation of the blood took place.

The less pronounced fibrinolytic activity in the maternal blood, and particularly in the cord blood, following elective Caesarean section compared with the findings following vaginal delivery may well be due to the more gentle manner of delivery.

That the cyclopropane anaesthesia did not cause changes in coagulation or fibrinolysis factors is in conformity with the studies of *Howland et al* (1959) and *Vanderveen et al* (1962). However the former found increased fibrinolysis in half the patients, but only after major long-lasting operations.

### SUMMARY

A study of the coagulation and fibrinolysis factors immediately post partum in women delivered by elective Caesarean section and their infants showed compared with normal mothers and their infants

A. Maternal blood. Less marked clot-promoting activity in the plasma. Otherwise no major changes in the coagulation or fibrinolysis factors.

B. Cord blood. Prolonged recalcification time and no clot promoting components in the plasma as are invariably present following vaginal delivery.

A reduced fibrinolytic activity.

### REFERENCES

- Howland W S, Zucker M B, Clifton E E and Boyan C P* *Anesthesiology* 20:28 1959  
*Masure R and Schou e R* *Brux-méd* 36:1434 1956



- Nielsen, N. C., *Acta obstet. gynec. scand.* 48: 371, 1969 a  
Nielsen, N. C., *Acta obstet. gynec. scand.* 48: 392, 1969 b  
Schwartz, C. L. J. *Obstet. Gynec. Brit. Emp.* 58: 538, 1951  
Vanderwee, J. L., McGovern, J. J., Barker, J. P. and Goldstein, R. *Anesthesiology* 23: 92, 1962

Received on Nov. 28, 1968

Incidentally elective Caesarean section does not appear to cause major changes in maternal coagulation factors. The finding that the values for PP (186 per cent) was lower than following vaginal delivery (PP 227 per cent) does not explain, but presumably suggests inaccuracy of the method, the determination being based upon dilution curves plotted on logarithmic paper where the uncertainty is most marked in the high concentrations.

The flatter course of the TAT two-stage and the prolonged recalcification time in cord blood following elective Caesarean section accord with the fact that no activation of the blood took place

The less pronounced fibrinolytic activity in the maternal blood, and particularly in the cord blood following elective Caesarean section compared with the findings following vaginal delivery may well be due to the more gentle manner of delivery

That the cyclopropane anaesthesia did not cause changes in coagulation or fibrinolysis factors is in conformity with the studies of *Howland et al* (1959) and *Vanderveen et al* (1962). However the former found increased fibrinolysis in half the patients, but only after major long-lasting operations

## SUMMARY

A study of the coagulation and fibrinolysis factors immediately post partum in women delivered by elective Caesarean section and their infants showed, compared with normal mothers and their infants

A. Maternal blood Less marked clot-promoting activity in the plasma. Otherwise no major changes in the coagulation or fibrinolysis factors

B. Cord blood Prolonged recalcification time and no clot promoting components in the plasma as are invariably present following vaginal delivery

A reduced fibrinolytic activity

## REFERENCES

- Howland W S, Zucker M B, Clifton E E and Boyan C P* *Anesthesiology* 20:28 1959  
*Masure R and Schonne R* *Brux-méd.* 36:1434 1956

Nielsen, N. C., *Acta obstet. gynec. scand.* 48: 371, 1969 a

Nielsen, N. C., *Acta obstet. gynec. scand.* 48: 392, 1969 b

Schwabler C. L. *J. Obstet. Gynec. Brit. Emp.* 58, 538, 1951

Vanderveen J. L., McGovern J. J., Bunker J. P. and Goldstein R. *Anesthesiology* 23: 92, 1962

Received on Nov. 28, 1968

Incidentally elective Caesarean section does not appear to cause major changes in maternal coagulation factors. The finding that the values for PP (186 per cent) was lower than following vaginal delivery (PP 227 per cent) does not explain, but presumably suggests inaccuracy of the method the determination being based upon dilution curves plotted on logarithmic paper where the uncertainty is most marked in the high concentrations.

The flatter course of the TAT two-stage and the prolonged recalcification time in cord blood following elective Caesarean section accord with the fact that no activation of the blood took place.

The less pronounced fibrinolytic activity in the maternal blood, and particularly in the cord blood, following elective Caesarean section compared with the findings following vaginal delivery may well be due to the more gentle manner of delivery.

That the cyclopropane anaesthesia did not cause changes in coagulation or fibrinolysis factors is in conformity with the studies of *Howland et al* (1959) and *Vanderveen et al.* (1962). However the former found increased fibrinolysis in half the patients, but only after major long lasting operations.

## SUMMARY

A study of the coagulation and fibrinolysis factors immediately post partum in women delivered by elective Caesarean section and their infants showed compared with normal mothers and their infants

A. Maternal blood Less marked clot-promoting activity in the plasma. Otherwise no major changes in the coagulation or fibrinolysis factors.

B. Cord blood Prolonged recalcification time and no clot promoting components in the plasma as are invariably present following vaginal delivery.

A reduced fibrinolytic activity.

## REFERENCES

- Howland W S, Zucker M B, Clifton E. E and Boyan C P* *Anesthesiology* 20: 28 1959  
*Masure R. and Schonne R.* *Brux.-méd.* 36: 1434 1966

(77.5 percent) represented treatment with Conluton® (Norethindron 2 mg + Mestranol 0.1 mg). In order to collect as accurate data as possible every woman was given a form on which to note the day of the last withdrawal bleeding and then the two or three first spontaneous menstrual periods. The participants were then asked to mail the form to the author and were informed that when it had been received, a prescription for contraceptive pills for 6 treatment cycles would be available at a drug store. If the form was not received in reasonable time the women were contacted by telephone or by mail. One of the patients could not be traced and was therefore not included in the analysis.

The patients were divided into three groups depending upon their menstrual patterns before the contraceptive therapy. Some of the participants had some difficulty in describing their usual menstrual cycle adequately.

Group I (432 patients) consisted of those who knew or believed that their menstrual cycles had a duration of about one month with a maximum deviation of one week between the shortest and the longest cycles.

Group II (76 patients) consisted of those who had irregular periods.

Group III included those who had regular periods with a duration of about 3 weeks or less or of 5 weeks or more. This group consisted of 7 subjects and was excluded from further analysis.

Thus, the remaining material consisted of 508 patients. Table I shows the number of individuals in the two remaining groups divided into age groups. The mean age in the whole series was 29.9 years.

### Results

Figs. 1 and 2 show the distribution of the length of the first three post treatment cycles in Group I and Group II.

Table II gives the mean length of these cycles in the two groups.

Table III shows the percentage of the first three cycles that were less than 36 days and less than 71 days respectively.

## THE LENGTH OF THE FIRST THREE MENSTRUAL CYCLES AFTER COMBINED ORAL CONTRACEPTIVE TREATMENT

BY

ULF LARSSON-COHN

Several reports (Shearman 1966 and 1968 Whitelaw et al., 1966 Rice Wray et al. 1967) have appeared of persisting amenorrhoea after oral contraceptive therapy. This communication describes the time interval between the last withdrawal bleeding and the first, second and third spontaneous menstrual flow in 516 women who had used combined oral contraceptives for periods between 9 and 55 months.

### *Material and Methods*

The material consisted of 516 women who visited the out-patient clinic of the Department of Obstetrics and Gynaecology at the University Hospital of Uppsala in order to get a renewal of their prescriptions for oral contraceptives. The first 300 patients were recommended to withhold their medication until they had had two spontaneous menstrual periods. In the latter part of the investigation, all new participants were asked to await three menstrual periods before they restarted the therapy. This second group consisted of 216 women.

All patients had used combined drugs only. The duration of the therapy was between 9 and 55 months with a mean of 22.5 months. Most combined oral contraceptive drugs available on the Swedish market had been used but the majority of the cycles

Table I. Number of Women in Group I (Regular Periods) and Group II (Irregular periods) Divided into Age Groups

Age in Years	Group I	Group II
17-20	20	1
21-30	253	52
31-40	140	21
41-49	19	2

Table II. Mean Duration in Days of the First Three Post Treatment Cycles in Group I (Regular Periods) and Group II (Irregular Periods)

	Group I	Group II
First post-treatment cycle	36.0	41.0
Second post treatment cycle	29.9	36.8
Third post-treatment cycle	29.6	37.3

Table III. Percent 50 of the First Three Post Treatment Cycles that Were Less than 35 and 70 Days Respectively

		Group I	Group II
First post-treatment cycle	≥ 35 day	50.3	33.4
	≥ 70	98.2	94.9
Second post-treatment cycle	≥ 35 day	88.6	51.3
	≥ 70	99.7	93.3
Third post treatment cycle	≥ 35 day	88.0	48.9
	≥ 70	99.5	88.5

Seventeen pregnancies occurred during the observation time, 2 of these during the first post-treatment cycle and 12 during the second. Seven of these subjects declared that they had tried to get pregnant and 7 that they had tried to avoid pregnancy. It was not possible to get a direct answer in 3 cases.

Four patients, one of which belonged to Group I and three

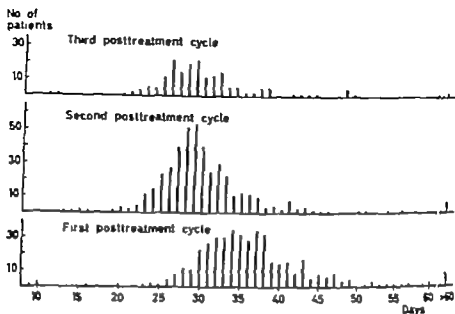


Fig. 1 Duration of the first three post treatment cycles among the patients who had regular periods before treatment.

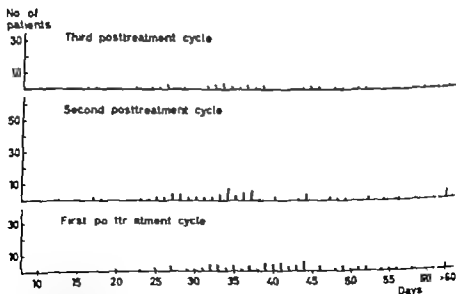


Fig. 2. Duration of the first three post treatment cycles among the patients who had irregular periods before treatment



There is reason to believe that the same variability existed in the present series, a circumstance that must be kept in mind when the results are discussed. This fact, however, does not exclude the validity of dividing the material into the two groups described earlier. It is reasonable to assume that those patients who declared that their cycles usually had a length of about 28 days differed statistically from those who said that their periods used to be irregular.

This study shows that in most subjects who usually have regular periods, the first menstrual cycle after combined oral contraceptive therapy is longer than the following ones. The mean difference is about 6 days with a considerable variation in both directions.

Bell and Loreane (1967) studied 10 subjects during the first and second cycle after combined oral contraceptive therapy. Their conclusion was that the variations from normality in respect of steroid and gonadotrophin output were relatively minor and mainly confined to the first post-treatment cycle.

This investigation shows that in women with regular periods the pre-treatment menstrual pattern is usually restored by the second post-treatment cycle—a fact that might be explained by the study of Bell and Loreane. Rice-Wray *et al.* (1967) studied a group of 125 women who had used combined drugs for up to 76 cycles. They found that in 2 of their cases the first post-treatment cycle was longer than 180 days. However, no information was given about the menstrual patterns before the therapy.

In the present study 4 out of 515 subjects developed amenorrhoea lasting more than 180 days. It is noteworthy that 3 of these 4 women had had irregular menstrual periods before the therapy. The main conclusion of this investigation is therefore that combined oral contraceptive agents should be used with caution in women who have irregular menstruation, especially in those who have not completed their family.

Rice-Wray *et al.* also studied a group of 38 women who had used sequential regimens and found a higher percentage of first post-treatment cycles of normal length (24–35 days) than among those who had used combined drugs. As the series was very limited, it is doubtful if any valid conclusion may be drawn from

who belonged to Group 2 did not get their first spontaneous menstrual bleeding within 6 months. They are here described in detail

*Case 1* Unmarried university student 26 years old. Before therapy periods always regular with a duration of about 28 days. An illegal abortion at 23 years of age. Took contraceptive tablets during 26 cycles after which she had an amenorrhoea of 9 months duration. She was then referred to the hospital for a complete endocrinological investigation. Biopsy showed a proliferative endometrium. There was a relatively low excretion of FSH and LH. After stimulation with human FSH she showed with a marked increase of total oestrogens in the urine. The case was interpreted as a persisting hypothalamic pituitary depression.

*Case 2* Unmarried 24 years old university student who had never been pregnant. Always irregular bleeding with a cycle length usually between 4 and 7 weeks. Had taken contraceptive tablets for 24 cycles. The first spontaneous menstrual period which was described as fairly normal, occurred 4 months after her last withdrawal bleeding. She had no further menstrual period during the following 5 months. After that time she left Sweden and was lost for follow-up.

*Case 3* Pharmacist, unmarried 23 years old. Had never been pregnant. Always irregular cycle with a duration usually between 1 and 2 months. Oral contraceptives for 2 years. After that amenorrhoea for 11 months, because of which she was scheduled for an endocrinological investigation. She was however in the meantime prescribed gestagenic hormones cyclically by another gynaecologist. As these hormones gave her regular bleeding she refused to be admitted for investigation.

*Case 4* Married 29 years old with 2 children and one therapeutic abortion. Irregular menstruation after the birth of the second child. This tendency was more pronounced after the abortion. She was prescribed oral contraceptives after amenorrhoea of more than one year's duration and took the tablets for 2 years. The first post treatment cycle had a duration of 39 days after which she developed amenorrhoea that still persisted 10 months later. She is scheduled for an endocrinological investigation.

### Discussion

Treolar *et al* (1967) and Chlax *et al* (1968) showed in their large prospective studies that in their series the majority of women had a considerable variation in the length of their menstrual cycles although most of them thought that their periods were regular. In the age group 25-34 years for example 20 per cent of the cycles had a duration of less than 25 days or more than 32 days.

## PRIMARY SARCOMA OF THE OVARY

BY

USKO NIEMINEN, CLAES VON NUMERS AND ESKO PUROLA

Primary sarcoma of the ovary is one of the least common gynaecological neoplasms. Miller (1937) reviewed 25 series comprising a total of 12,092 ovarian tumours, of which 343 were ovarian sarcomas (2.84 per cent). In a series of 231 ovarian tumours Vrejolu and Manolescu (1964) detected only three sarcomas (1.3 per cent). According to Novak and Woodruff (1967) the ratio of sarcoma of the ovary as compared to carcinoma is about one to 40. Both Haines and Taylor (1962) and Vrejolu and Manolescu maintain that in those series reporting an exceptionally high ratio of sarcoma, other types of tumour—e.g. thecoma, granulosa cell tumour, dysgerminoma, arrhenoblastoma—has been erroneously diagnosed as sarcoma. In addition, Haines and Taylor point out that sarcomatous areas may be predominant in a malignant teratoma, and that oedematous, haemorrhagic or degenerate fibromas may be confused with sarcoma.

Ovarian sarcoma is encountered in all age groups, but in contrast to other malignant tumours it has been found in exceptionally young females. In Miller's survey the age groups 21–30 and 41–50 predominated, but 15 per cent of the patients were aged 11–20 years and about 4 per cent were under 10 years old. Occasional cases of sarcoma in fetuses and infants have been reported.

Ovarian sarcoma may be bilateral and this was found in 24.9 per cent cases in Miller's review.

On the basis of the microscopic structure, ovarian sarcoma may

their investigation. In the absence of more reliable data, it is nevertheless possible that the sequential regime should be preferred when oral contraceptives are prescribed for women with irregular menstrual periods especially those who have not completed their childbearing period.

### SUMMARY

The length of the first three menstrual cycles after combined oral contraceptive therapy was investigated in 516 women. The material was divided into those who had regular (Group I) and those who had irregular bleeding (Group II) before the therapy.

In Group I the first post treatment cycle was about 6 days longer than the following ones which had about the same duration as before the therapy. Four patients developed amenorrhoea that persisted for more than 6 months. Three of these patients belonged to Group II. It is recommended that combined oral contraceptives should be prescribed with caution to women with irregular menstrual periods who have not completed their childbearing period.

### REFERENCES

- Bell E T and Loaine J A. *Lancet* 2 44 1967  
Chiazze L, Brayer F T, Macisco J J, Parker M. P and Duffy B. J. *JAMA* 203 377 1968  
Rice-Wray E, Correu S, Gorodorsky J, Esquituel J and Goldzleher J W. *Fertil Steril* 18 712 1967  
Shearman R. P. *Lancet* 2 1110 1966  
Shearman R. P. *Lancet* 1 325 1968  
Treolar A. E., Boynton R. E., Belin B. G. and Brown B. W. *Int. J. Fertil* 12 77 1967  
Wittelsoe M. J., Nola V. F. and Kalman G. F. *JAMA* 195 780 1966

Received on May 2 1968

*Laryssa, Udo, von Numer, Claës and Purola, Eds, Acta obst. et gynec. scandinav. 48, 421, 1969*  
From the Departments I and II of Obstetrics and Gynecology (Professor  
Aaro Tarkenton M.D. and Professor Paavo Vasa, M.D.) University Central  
Hospital, Helsinki Finland

## PRIMARY SARCOMA OF THE OVARY

BY

USKO NIBAINEN, CLAËS VON NUMER AND ESKO PUROLA

Primary sarcoma of the ovary is one of the least common gynaecological neoplasms. *Miller* (1937) reviewed 25 series comprising a total of 12,092 ovarian tumours, of which 343 were ovarian sarcomas (2.84 per cent). In a series of 231 ovarian tumours *Vrejtou and Manolescu* (1964) detected only three sarcomas (1.3 per cent). According to *Novak and Woodruff* (1967) the ratio of sarcoma of the ovary as compared to carcinoma is about one to 40. Both *Haines and Taylor* (1962) and *Vrejtou and Manolescu* maintain that in those series reporting an exceptionally high ratio of sarcoma, other types of tumour—e.g. thecoma, granulosa cell tumour, dysgerminoma, arrhenoblastoma—have been erroneously diagnosed as sarcoma. In addition, *Haines and Taylor* point out that sarcomatous areas may be predominant in a malignant teratoma, and that oedematous, haemorrhagic or degenerate fibromas may be confused with sarcoma.

Ovarian sarcoma is encountered in all age groups, but in contrast to other malignant tumours it has been found in exceptionally young females. In *Mills's* survey the age groups 21–30 and 41–50 predominated, but 15 per cent of the patients were aged 11–20 years and about 4 per cent were under 10 years old. Occasional cases of sarcoma in foetuses and infants have been reported.

Ovarian sarcoma may be bilateral and this was found in 24.9 per cent cases in *Miller's* review.

On the basis of the microscopic structure, ovarian sarcoma may

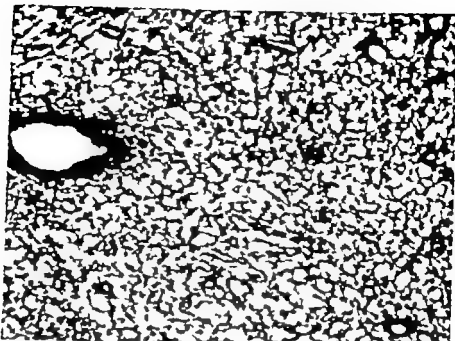


Fig. 1 Case 1 Reticular tumour tissue rich in cells in structure resembling endometriotic stroma tissue  
Haemat. + van Gieson  $\times 240$

be classified in various ways. The nomenclature is noticeable inconsistent and varied. Miller suggested a division into anaplastic or poorly differentiated tumours on the one hand and well differentiated neoplasms on the other. In the former group he included round cell spindle cell and giant cell tumours, and also melanosarcomas in the latter fibroblastic lipoblastic, chondroblastic osteoblastic and myoblastic tumours and adenosarcomas. In addition, *Balds* and *László* (1965) cited descriptions of ovarian tumours classified as leiomyo- rhabdomyo- carcino- and neurofibrosarcoma, and *Novak* and *Woodruff* mentioned angiosarcoma. It seems possible that the tumours in question were teratomatous.

According to *Novak* and *Woodruff* an ovarian sarcomatous tumour is generally macroscopically moderate in size and as a rule lobulated or nodular. The cut surface is firm in some places but there is invariably a tendency towards necrosis with the production of soft pultaceous areas or ragged cystic cavities.

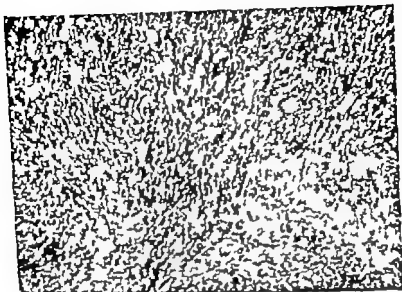


Fig. 2. Case 2. Slightly differentiated fibrosarcoma.  
Haematoxylin-Gieson  $\times 120$ .

### Material

During the years 1945-1966 a total of about 2800 patients with benign ovarian neoplasms, and 1090 with malignant primary ovarian neoplasms were treated at Departments I and II of Obstetrics and Gynaecology Helsinki University Central Hospital. Five of these tumours, detailed in this paper, were ovarian sarcomas. The frequency was thus 0.16 per cent of the total series and 0.46 per cent of the malignant cases. Originally a diagnosis of ovarian sarcoma was made in 16 cases, but some of these were secondary tumours and in others the diagnosis was altered on re-evaluation of the specimens.

*Case 1.* A 55-year-old unmarried nulliparous woman had left oophorectomy performed on August 18, 1950. The uterus and the right adnexa were normal. The left ovary was replaced by a tumour about 8 cm in diameter, smoothly rounded in outline, which was extensively adherent to the sigmoid colon — Pathologist's report: The specimen shows solid tumour tissue forming exten-

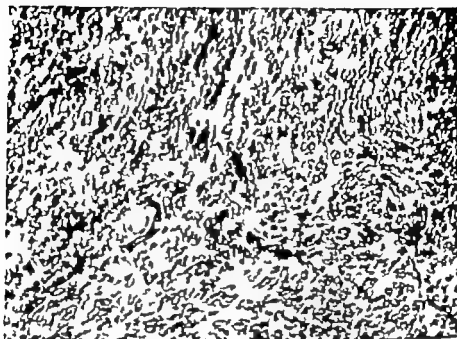


Fig. 3 Case 2. Same tumour tissue as in Fig. 2.  
Haemat. + van Gieson.  $\times 740$

are irregularly shaped areas. Between these without any sharp demarcation from the tumour tissue there is stroma composed of connective tissue poor in cells. The tumour tissue is highly cellular slightly polymorphocellular and in parts clearly reticular in structure. Mitoses are scanty. The structure of the tumour undoubtedly justifies a diagnosis of sarcoma of low grade malignancy originating in stromal endometriosis. (Fig. 1) —Postoperatively the patient was given radium and radiation therapy. She was last seen on March, 1959. No recurrence or changes indicative of metastases were then observed. The patient died on Sept. 10, 1960 from cardiac infarction and diabetes.

**Case 2.** A 48-year-old nulliparous married woman had bilateral salpingo-oophorectomy performed on Nov. 11, 1952. In addition, resection of the ileum and bladder was carried out. From the left ovary a tumour extended to two finger-breadths below the level of the umbilicus. It ruptured on removal, and a greenish thin fluid containing necrotic pieces of tissue flowed out. —*Pathologist's report.* The tumour tissue shows infiltrative growth and is highly cellular. The cells are relatively small and their nuclei are slender or rounded. Occasional fibres occur in the tumour tissue. Mitoses are found in moderate numbers. The tumour is a slightly differentiated fibrosarcoma. (Figs. 2 and 3) —Postoperatively the patient received radiation therapy. She died from extension of the ovarian tumour on Feb. 19, 1954.

**Case 3.** A 50-year-old married woman had four children and no abortions.



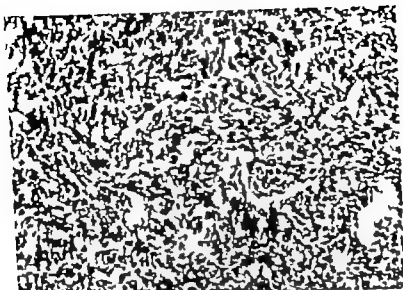


Fig. 4 Case 3 Polymorphocellular fibrosarcomatous tissue  
H&E + in Giemsa  $\times 240$

Subtotal hysterectomy and bilateral salpingo-oophorectomy were carried out on Sept. 3, 1953. There was a nodular encapsulated tumour about 7 cm in diameter arising from the left ovary. The cut surface of the tumour was pale and homogeneous.—*Pathologist's report* The specimen shows highly cellular tumour tissue of mesenchymal type. In parts the nuclei are rather long and slender in parts rounded, polymorphous, varying in chromatin concentration. Mitoses are seen in abundance. By means of lipid staining, which was negative, the possibility of theca-cell tumour is eliminated. The tumour is fibrosarcoma, variable in degree of differentiation (Fig. 4). Postoperatively the patient was given radiation therapy. She died from her ovarian tumour on Oct. 6, 1964.

**Case 4** A 70-year-old married woman had four children and no abortions. Subtotal hysterectomy and bilateral salpingo-oophorectomy were carried out on May 4, 1959. In addition, appendectomy was performed. From the left ovary fluctuant tumour with dark surface and smooth rounded outline extended 5 cm above the umbilicus. The tumour weighed 5570 g. When bisected it was found to be a multilocular cyst, containing old blood.—*Pathologist's report* In specimens from those parts of the tumour wall which contain relatively small cysts, cavities of varying size occur lined by simple columnar or cuboidal epithelium. In the non-cystic parts the tumour wall consists of two different layers of tissue: an outer layer made up of dense

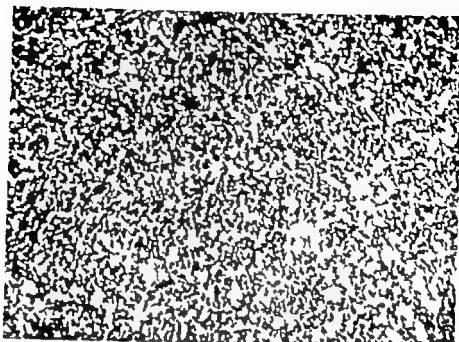


Fig. 5. Case 4 Highly cellular tumour tissue, composed of atypical smooth muscle tissue

Haemat. + van Gieson.  $\times 120$

connective tissue and an inner layer in some places of considerable thickness, made up of relatively closely packed and moderately pleomorphic cells. The cells are either fusiform in shape or more or less stellate with numerous anastomosing protoplasmic processes. Mitoses occur in relative abundance. The tissue is undoubtedly atypical smooth muscle tissue. The microscopic examination suggests a diagnosis of serous ovarian cystadenoma with benign epithelial components and atypical and obviously malignant smooth muscle tissue infiltrating the wall. (Figs. 5 and 6) Postoperatively the patient received radiation therapy. She was still alive on Jan. 12, 1966.

**Case 5** A 62 year-old married woman with no data available regarding parity underwent exploratory laparotomy on Jan. 29, 1963. The lower part of the abdomen was filled by an inoperable tumour some 30 cm in diameter. When removal was attempted the tumour ruptured and serous fluid flowed out. The tumour was in part cystic.—*Pathologist's report* The specimen shows richly vascularized polymorphocellular tumour tissue clearly reticular in structure. The tumour cells anastomose by plasma processes some of which are noticeably long. The nuclei show a wide variation in size and shape. Multinucleated giant cells occur. Mitoses are abundant. The reticular structure seems to be indicative of a tumour originating in endometrial stroma tissue.

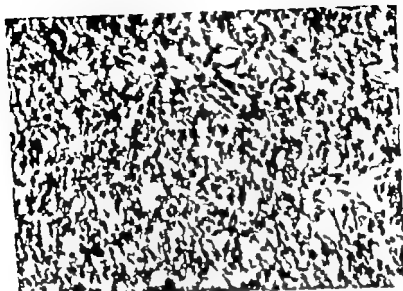


Fig. 6 Case 4. Same tumour tissue as in Fig. 5.  
Haematoxylin and Eosin  $\times 240$

However, owing to the marked atypia and polymorphism this assumption remains open in doubt (Figs 7 and 8).—Postoperatively the patient received radiation therapy. She died on May 9 1963, from ovarian tumour.

### Discussion

Primary ovarian sarcoma is indeed a rare tumour, as is borne out by the incidence found in this series. This supports the impression that reports indicating an exceptionally high frequency arise from errors in interpretation of the specimens. In our hospital a total of 16 ovarian sarcomas were diagnosed in 20 years. After thorough perusal of the hospital records and re-evaluation of the histological specimens only five cases proved to be primary ovarian sarcoma.

As compared to previous reports the mean age of our patients (57 years) was relatively high. The youngest patient was 48 and the oldest 80. All cases were diagnosed after the menopause.

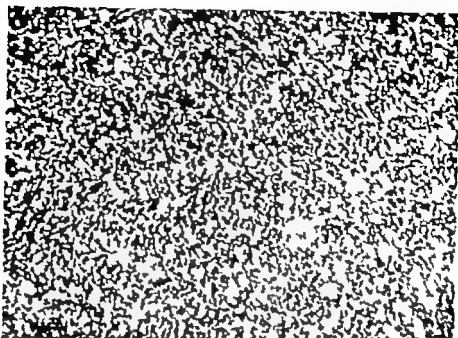


Fig. 5. Case 4. Highly cellular tumour tissue composed of atypical smooth muscle tissue.

Haemat. + van Gieson,  $\times 170$ .

connective tissue and an inner layer in some places of considerable thickness, made up of relatively closely packed and moderately pleomorphic cells. The cells are either fusiform in shape or more or less stellate with numerous anastomosing protoplasmic processes. Mitoses occur in relative abundance. The tissue is undoubtedly atypical smooth muscle tissue. The microscopic examination suggests a diagnosis of serous ovarian cystadenoma with benign epithelial components and atypical and obviously malignant smooth muscle tissue infiltrating the wall. (Figs. 5 and 6) Postoperatively the patient received radiation therapy. She was still alive on Jan. 1., 1966.

Case 5. A 61-year-old married woman with no data available regarding parity underwent exploratory laparotomy on Jan. 29 1963. The lower part of the abdomen was filled by an inoperable tumour some 70 cm in diameter. When removal was attempted the tumour ruptured and serous fluid flowed out. The tumour was in part cystic.—*Pathologists' report.* The specimen shows richly vascularized polymorphocellular tumour tissue clearly reticular in structure. The tumour cells anastomose by plasma processes some of which are noticeably long. The nuclei show a wide variation in size and shape. Multinucleated giant cells occur. Mitoses are abundant. The reticular structure seems to be indicative of a tumour originating in endometrial stroma tissue.

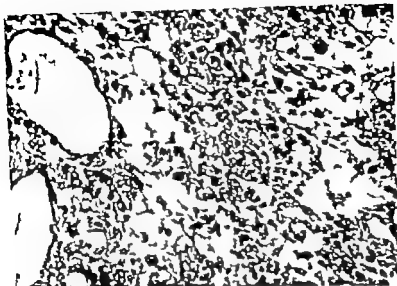


Fig. 8 Case 5 Same tumour tissue as in Fig. 7  
Hæmst. on Gieson 240

Baldes and Lázló (1965) have each described one case of leiomyosarcoma. Izze (1951) described four cases, in three of which the presence of myogenic elements was an outstanding histological feature. In contrast to this type of myosarcoma, rhabdomyosarcoma has been reported several times.

In one case (no. 1) the sarcoma obviously originated in ovarian endometriosis, and in a further case (no. 5) the genesis was probably the same. Benjamin and Campbell (1960) described one such case and mentioned that they had not seen any previous similar report. Sarcoma originating in endometriotic tissue in other parts of the genital tract, the uterus in particular, is a more familiar phenomenon. About 100 such cases of stromal endometriosis have been reported in the English literature (Stearns, 1958). Stromal endometriosis, also called atromatous endometriosis, atromatosis and stromal adenomyosis, has been recognized and described as an entity for many years, but its histogenesis, prognosis and possible relationship with endometriosis and endometrial sarcoma are not yet fully understood.

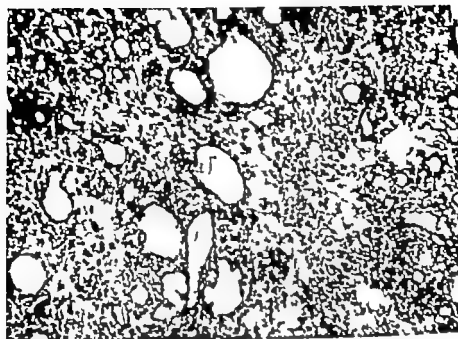


Fig 7 Case 5 Reticular polymorphocellular sarcomatous tissue.  
Haemat. + van Gieson.  $\times 100$

However as children are not admitted to our hospital this age group remains outside the scope of this study

All the present cases were unilateral. All tumours were relatively large. The smallest one was about 7 cm in diameter and the largest filled almost the whole of the abdominal cavity.

Three of the patients have subsequently died from ovarian sarcoma. One died from cardiac infarction ten years after operation and one is still alive.

In two cases (nos. 2 and 3) the tumour was a fibrosarcoma, which is not considered as an unusual type of ovarian sarcoma. Giant follicular lymphoid hyperplasia morbus Brill Symmers, (Schröder 1962) sometimes develops in association with ovarian fibrosarcoma, with enlargement of lymph nodes in various parts of the body. Glandular enlargement was not observed in our two cases.

In one case (no. 4) the structure of the tumour was that of leiomyosarcoma. This case has previously been described by von Numers and Mikkonen (1960) Kelley and Scully (1961) and

From the Departments of Endocrinology and Metabolism (Prof R. Lufs) and the Department of Pathology (Prof B. Thorell) Karolinska Sjukhuset Stockholm Sweden

## LEYDIG CELL TUMOURS OF THE OVARY

Report of Three Cases

BY

ERIK ALLANDER AND IAN WÄGERMARK

Leydig cell tumour of the ovary is a rare usually virilizing tumour. Other ovarian and adrenal tumours can also be virilizing and the main problem for the clinician is to localize the source of the excessive hormone production. The morphologist on the other hand, has to face the fact that histologically identical tumours may differ considerably in their endocrine activity. All these facts have caused confusion, and a number of partially or completely synonymous terms have appeared in literature.

On the basis of three cases the clinical and morphological problems will be discussed with emphasis on ontogenetic and biochemical aspects.

**Case 1** Woman born in 1903. No family history of diabetes. II-grav II-para, menarche at the age of 14 and menopause at the age of 52 (1955). Hypertrichosis and acromegaly since 1960. In 1959 hypertrichosis was noticed on face and neck. When admitted to hospital in 1963 the patient had a male hair pattern, facial hypertrichosis and hypertrophy of the clitoris. Distribution of pubic hair was normal. The appearance was not typical of Cushing's syndrome.

Laboratory tests showed normal glucose tolerance and repeatedly normal values for urinary 17-ketosteroids (17-KS) and 17-sterogenic steroids (17-RGS) (4-7 mg and 16-18 mg/24 h). No reduction of 17-KS in dexamethasone suppression test (8 mg) but normal reduction of 17-RGS. An ovarian tumour

## SUMMARY

The literature on ovarian sarcoma is reviewed. Five primary ovarian sarcomas are described which were discovered in a series of 3890 operatively treated ovarian tumours. On the basis of their histological structure two were classified as fibrosarcomas. In one case the neoplasm was a very rare mixed tumour with leiomyosarcomatous tissue elements in the wall of a benign serous cystadenoma. Sarcoma originating in an ovarian endometriotic focus was obviously present in one case and probably in another. Only one similar case has previously been described.

## REFERENCES

- Baldz M and László J. *Zentralbl. Gynäk.* 87 633, 1965  
Benjamin F and Campbell J. *Am. J. Obst. & Gyn.* 80 449, 1960  
Haines E. and Taylor W. *Gynaecological Pathology*—Churchill London 1962  
Istre B. *J. Oslo City Hosp.* 10 231 1951  
Kelley R. and Scully R. *Cancer* 14 939 1961  
Miller J. *Handbuch der speziellen pathologischen Anatomie und Histologie* VII Band, III Teil, Eierstock. Julius Springer Berlin 1937  
Novak E. and Woodruff J. *Novak's Gynecologic and Obstetric Pathology* 6th Ed. Saunders Philadelphia 1967  
von Numers C. and Mikkonen R. *Ann. Chir. et Gynaec. Fenn.* 49 240, 1960  
Schröder F. *Zentralbl. Gynäk.* 84 1754 1962  
Stearns H. *Am. J. Obst. & Gyn.* 75 663 1958  
Vrejole Gh. and Manolescu N. *Prosectura Spital. Coltea Bucuresti* 3 209 1964  
Cit. Graur Gr. *Berichte Gynäk. Geburtsh.* 88 214 1965

Received on July 10 1968



From the Department of Endocrinology and Metabolism (Prof R. Lef1) and the Department of Pathology (Prof B. Thorell) Karolinska Sjukhuset Stockholm Sweden

## LEYDIG CELL TUMOURS OF THE OVARY

Report of Three Cases

BY

ERIK ALLANDER AND JAN WÄGERMARK

Leydig cell tumour of the ovary is a rare usually virilizing tumour. Other ovarian and adrenal tumours can also be virilizing and the main problem for the clinician is to localize the source of the excessive hormone production. The morphologist, on the other hand, has to face the fact that histologically identical tumours may differ considerably in their endocrine activity. All these facts have caused confusion, and a number of partially or completely synonymous terms have appeared in literature.

On the basis of three cases the clinical and morphological problems will be discussed with emphasis on ontogenetic and biochemical aspects.

**Case 1** Woman born in 1903. No family history of diabetes. II-grav II-para, menarche at the age of 14 and menopause at the age of 52 (1955). Hypertension and angina pectoris since 1960. In 1959 hypertrichosis was noticed on face and neck. When admitted to hospital in 1963 the patient had male pattern alopecia (facial hypertrichosis and hypertrophy of the clitoris). Distribution of pubic hair was normal. The appearance was not typical of Cushing's syndrome.

Laboratory tests showed normal glucose tolerance and repeatedly normal values for urinary 17-ketosteroids (17-KS) and 17-ketogenic steroids (17-KGS) (4-10 mg and 10-18 mg/4 h). No reduction of 17-KS in dexamethasone suppression test (8 mg) but normal reduction of 17-KGS. An ovarian tumour

was considered most probable and at operation the right ovary was found to be somewhat enlarged. It contained a well-defined tumour with a diameter of 1.5 cm situated in the hilar region.

Microscopically the tumour consisted of cells with abundant eosinophilic cytoplasm, several containing so called Reinke crystalloids. The nuclei were distinctly outlined, had a loose network of chromatin and often a prominent nucleolus. The cells were closely packed without any special arrangement. *Diagnosis* Leydig cell tumour

In January 1967—four years after the operation—the virilization had regressed but the patient still had moderate facial hypertrichosis. Her voice was now normal and the clitoris of ordinary size. Laboratory values for 17 KS and 17 KGS were normal (4.3 and 14.4 mg/24 h)

*Case II* Woman born in 1897 Diabetic mother 0-grav., menopause at the age of 49 (1946) Diabetes since 1954 well controlled with oral hypoglycaemic agents (chlorpropamide) In 1958 the patient began to become bald and facial hypertrichosis appeared. On her legs and back the growth of hair increased When admitted to hospital in 1960 she was practically bald but had facial hypertichosis. Normal pubic hair and no hypertrophy of the clitoris or Cushingoid appearance There were normal values for urinary 17 KS and 17 KGS (5–9 mg and 16–18 mg/24 h) On dexamethasone suppression test (8 mg) no reduction of 17 KS on the other hand a normal reduction of 17 KGS

An ovarian tumour was suspected clinically and a bilateral oophorectomy was performed. The left ovary contained a tumour with a diameter of 1 cm in the hilar region. The right ovary contained a somewhat larger cystic tumour Histological examination showed that the tumour from the left ovary was made up of polygonal cells with fairly large pale nuclei with distinct nucleoli The cytoplasm contained lipochrome pigment and Reinke crystalloids In the surrounding tissue there were some islands of hyperplastic hilar cells. The wall of the cystic tumour in the right ovary consisted of similar tumour tissue Neither of the two tumours showed any signs of malignancy *Diagnosis* Bilateral Leydig cell tumour

Two years after the operation the scalp hair had begun to return, and the facial hypertrichosis decreased Values for urinary 17 KS 17 KGS oestrogens and gonadotrophines were normal for her age

*Case III* Woman born in 1903 No family history of diabetes 0-grav. menarche at the age of 14 and menopause at the age of 50 (1953) She has diabetes since 1965 and has been treated with insulin In 1958 baldness facial hypertrichosis and deepening of the voice were noticed In 1966 when she was admitted to hospital an increased growth of hair on her shoulders and back could also be noticed as well as a growth of pubic hair spreading up on the abdomen Moderate hypertrophy of the clitoris but not Cushingoid appearance

Laboratory tests showed normal values for cortisol in plasma, increased 17 KS and 17 KGS (16–36 mg and 15–28 mg/24 h) Tetrahydro E and tetrahydro F were 1.8 and 1.5 mg/24 h respectively Urinary pregnanetriol was

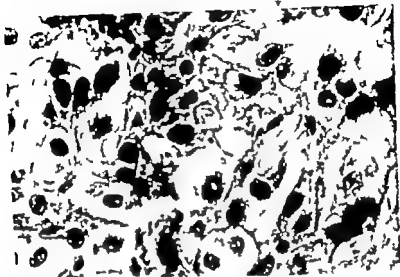


Fig 1 Tumour tissue composed of cells rich in pale, slightly eosinophilic cytoplasm. Note the big Reinke crystalloid in the right half of the picture 500 Case III

2.9 mg/24 h (upper borderline value) N suppression of 17 KS on Dexamethasone test (8 mg) not of 17 KGS. This raised the suspicion of an adrenal cortical tumour.

Radiographic examinations (angiography, coeliacography and pelvic arteriography) did not show any changes in the adrenals or in the region of the ovary. Transient asthma appeared after these arteriographies which were performed simultaneously.

A bilateral exploration of the adrenals was performed and on the right side a pea-sized tumour-like formation was found and removed. The left adrenal was normal. Microscopical examination of the right adrenal showed many tuberculous. No signs of TB in other organs could be found despite careful bronchial and abdominal examinations.

The search for the malignancy then had to be an ovarian tumour and at laparotomy was made. The lower part of the abdominal cavity was filled with mass originating from a benign ruptured mucocoele of appendix. The left ovary was apparently normal. The right ovary which was removed, contained tumour with diameter of 1.5 cm. Microscopically the tumour was similar to those of the other two patients and contained lipofuscin pigment and Reinke crystalloids (Fig 1). There were no signs of malignancy. The diagnosis was Leydig cell tumour.

### Discussion

Ovarian Leydig cell tumours are said to be rare and few cases have been published. As recently as 1959 there were only about 10 cases to be found in the literature (Merrill 1959). By 1966 the number had increased to 50 (Dunnihoo *et al.* 1966). However we have found reports of another two cases (Halinska *et al.* 1964 Marsiglia *et al.* 1965) we know about three which are being prepared for publication (Ernest personal communication) and have seen three ourselves. Evidently this tumour is not as uncommon as is generally believed.

**Morphology** For the morphologist the problem is to distinguish between tumours composed exclusively of closely packed "lipid cells". This group consists of Leydig cell tumours, adrenal rest tumours and the folliculome lipidique (Lecène) (see Morris *et al.* 1958). It is almost impossible to differentiate between these types. Only the tumours containing cells with the cytoplasmic bodies called Reinke crystalloids can be recognized as Leydig cell tumours. The presence of these crystalloids in Leydig cells is not usual. In normal testicular tissue they do not appear until after puberty and then are visible in only about 6 per cent of the Leydig cells (Gardener *et al.* 1957). With the electron microscope, however, it has been possible to demonstrate cytoplasmic structures of submicroscopical size which probably represent earlier phases in the formation of Reinke crystalloids.

**Function** If the morphology is similar within the group of lipid cell tumours the opposite is true of their function. The Leydig cell tumours, for example, are usually virilizing but cases with primarily oestrogenic effects have been described in the survey of Dunnihoo *et al.* (1966). This is easily understood since the intracellular synthesis of both androgens and oestrogens starts with progesterone. It has to be remembered that this substance is also the precursor of corticoids. The different pathways and their interrelationships for the synthesis of steroid hormones are well known from *in vitro* investigations (Besch *et al.* 1964 Kase *et al.* 1964 Lipsett *et al.* 1966 Samuels *et al.* 1967 Sandberg *et al.* 1962 Savard *et al.* 1961 Steinberger *et al.* 1967). The cells of the "lipid cell" tumours are potentially producers of steroid

hormones, and their enzyme complement determines which phases of the synthetic pathway will be dominant—and thereby also the clinical effects: virilization, feminization or Cushing syndrome (Gabrilove 1964).

**Ontogeny** The occurrence of adrenal (or adrenal rest) tumours in the ovaries is easily explained by the ontogenetic development of the gonads. These consist of three components and develop from the genital ridge, a thickening of the rim of the mesonephros. Their first component consists of a primitive mesenchyme. It is possible that the granulosa cells have their origin in this part. The surface of the ridge is covered by the second component, coelomic epithelium, from which the adrenal cortex is formed. The primordial sex cells, which constitute the third component, migrate to the area, probably from the intestinal region (see Morris *et al.* 1958).

The Leydig cells of the testicle as well as the so-called ovarian hilar cells differentiate from the primitive mesenchyme. These cells are normally to be found in the hilar region at the ovary in about 80 per cent of post-pubertal women (Gardener *et al.* 1957; Sternberg, 1949) and they constitute the origin of the Leydig cell tumours. It is worth noting that these tumours, often identical in structure and function, can be of mesenchymal as well as of epithelial origin.

**Suggestive Diagnostic Nomenclature** Our discussion has shown that it has to be difficult or impossible to distinguish morphologically between ovarian tumours composed solely of "lipid cells". In this we agree with a recently published paper by Taylor and Norris (1967). We believe that it would be both adequate and sufficient to present the morphological diagnosis as "potentially steroid-producing ovarian tumour" in addition to a judgement of the grade of malignancy based on the usual criteria. To that description available data on steroid production can be added, (e.g. benign potentially steroid-producing ovarian tumour testosterone-producing). According to current nomenclature of pathology (SNOP) (College of Amer Pathologists 1965) there is a possibility of making a diagnosis to different levels of precision using these principles.

**Clinical Diagnosis.** Since ovarian Leydig cell tumours practi-

### Discussion

Ovarian Leydig cell tumours are said to be rare and few cases have been published. As recently as 1959 there were only about 10 cases to be found in the literature (Merrill 1959). By 1966 the number had increased to 50 (Dunnihoo *et al.* 1966). However we have found reports of another two cases (Halinska *et al.* 1964, Marsiglia *et al.* 1965) we know about three which are being prepared for publication (Ernest personal communication) and have seen three ourselves. Evidently this tumour is not as uncommon as is generally believed.

**Morphology** For the morphologist the problem is to distinguish between tumours composed exclusively of closely packed "lipid cells". This group consists of Leydig cell tumours, adrenal rest tumours and the folliculome lipidique (Lecène) (see Morris *et al.* 1958). It is almost impossible to differentiate between these types. Only the tumours containing cells with the cytoplasmic bodies called Reinke crystalloids can be recognized as Leydig cell tumours. The presence of these crystalloids in Leydig cells is not usual. In normal testicular tissue they do not appear until after puberty and then are visible in only about 6 per cent of the Leydig cells (Gardener *et al.* 1957). With the electron microscope however it has been possible to demonstrate cytoplasmic structures of submicroscopical size which probably represent earlier phases in the formation of Reinke crystalloids.

**Function** If the morphology is similar within the group of "lipid cell" tumours the opposite is true of their function. The Leydig cell tumours for example are usually virilizing but cases with primarily oestrogenic effects have been described in the survey of Dunnihoo *et al.* (1966). This is easily understood since the intracellular synthesis of both androgens and oestrogens starts with progesterone. It has to be remembered that this substance is also the precursor of corticoids. The different pathways and their interrelationships for the synthesis of steroid hormones are well known from *in vitro* investigations (Besch *et al.* 1964, Kase *et al.* 1964, Lipsett *et al.* 1966, Samuels *et al.* 1967, Sandberg *et al.* 1962, Savard *et al.* 1961, Steinberger *et al.* 1967). The cells of the "lipid cell" tumours are potentially producers of steroid

## REFERENCES

- Beach, P. K. et al., *J Clin. Endocr* 24 1339 1964
- Committee on Nomenclature and Classification of Disease. College of Amer Pathologists. Systematized Nomenclature of Pathology Chicago 1965
- Dunshoo D. R. et al., *Obstet. Gynec.* 27 703 1966
- Ernest L., Personal communication
- Gabrilove J. L., *J Mount Sinai Hosp* 31 449 1964
- Gardner G. H. et al. *Amer J Obstet. Gynec.* 73 536, 1957
- Hakala A. et al. *Pol. Tyg. Lek.* 19 387 1964
- Kate H. et al. *Amer J Obstet. Gynec.* 90 1251 1964
- Lipsett M. B. et al. *Recent Progr Hormone Res.* 22, 245, 1966
- Margolis L. G. et al., *Rev Obstet. Gynec.* 25 671 1965
- Merrill, J. A., *Amer J Obstet. Gynec* 78 1254 1959
- Morris, J. M. et al., *Endocrine Pathology of the Ovary* The Mosby Co., St Louis, 1958
- Smaz, J. M. et al. *J Clin. Endocr* 27 615, 1967
- Sewald, L. T. et al., *Colloquia on Endocrinology* Vol 16 Ciba Foundation Churchill, 1967
- Saulberg, A. A. et al. *J Clin. Endocr* 22 929 1962
- Seward, K. et al. *J Clin. Endocr* 21 165, 1961
- Stalsberg, H. *Nord Med* 76 1021 1966
- Stamberger E. et al. *Colloquia on Endocrinology* Vol. 16, Ciba Foundation, Churchill, 1967
- Sternberg, W. H. *Amer J Pathol.* 25 453, 1949
- Taylor H. B. et al., *Cancer* 20 1953, 1967

Received on April 25 1968

cally always are small they will be diagnosed only when they produce hormones usually androgens. The clinical diagnosis in a female patient with virilism will be either ovarian or adrenal disease. It is well known that high as well as low values for the excretion of 17 KS and 17 KGS are seen in both ovarian and adrenal tumours. This is in accordance with the previous discussion which shows that biochemical analysis of the hormone production will give no information on the location of the tumour. Analysis of the steroid pattern in blood from the venous side of the respective organ might be tried (Saez *et al.* 1967).

Angiographic diagnosis is difficult and was not successful in our three cases. Retroperitoneal air insufflation is of limited value in revealing adrenal tumours.

In conclusion then it is clinically often impossible to differentiate between adrenal and ovarian virilizing "lipid cell" tumours, and a surgical exploration has to be performed. An analysis of blood testosterone levels and metabolism may be useful.

It is noticeable that among the 55 cases of ovarian Leydig cell tumours published up to now (our cases included) eleven were diabetics. The frequency of diabetes mellitus however is high in this age group but a connection between the two conditions might exist.

## SUMMARY

Three cases of virilizing Leydig cell tumours of the ovary are presented, and some of the diagnostic problems are discussed in connection with morphogenesis, morphology, steroid synthesis and the clinical picture. Difficulties appear when distinguishing morphologically between Leydig cell tumours and other lipid cell ovarian tumours and in differentiating clinically an ovarian tumour from one in the adrenal glands.

On the basis of data from morphology, function, ontogeny and clinic it is suggested that the description of the steroid producing group of ovarian tumours should be simplified according to current nomenclature (SNOP).



Table I. Reports on Cure Rates with Metronidazole

author	Year	Patients	Cure Rate
cookson et al.	1961	108	96 %
Arnold (combined therapy)	1961	140	89 %
Kinghley	1962	102	100 %
Svensson	1962	76	97 %
Bredland	1962	82	98 %
Casella	1963	93	92 %
Tolson and Treadwell	1963	2168 (summary of 48 studies)	98 %
Scott-Grey	1964	300 (pregnant women)	95 %
Gardner and Dolan	1964	244	97 %
Perry and Dee Lanning	1964	2002	98 %
Peri	1965	151 (pregnant women)	100 %
Parker et al.	1965	420	97 %
Peterson et al.	1966	208 (pregnant women)	99 %
Sando	1966	123 (pregnant women)	98 %
Arnold (combined therapy)	1966	72	76 %

Table II Age Distribution

15-19 years	13 patients
20-29 years	48 patients
30-39 years	28 patients
40-49 years	17 patients
50-59 years	14 patients
Total	120 patients

Table III Treatment Results with Metronidazole

	Patients	Not Cured	Cured	Cured after 2 Courses
Course I	120	45	73 (67 %)	
Course II	45	29	15 (34 %)	88 (73 %)

Two patients did not return for examination after course I

One patient did not return for examination after course II

## METRONIDAZOLE TREATMENT OF TRICHOMONAL VAGINITIS

A Comparison of Cure Rates in 1961 and 1967

J. CHR. AURE AND H. GJØNNÆSS

### *Introduction*

Until metronidazole was introduced as a trichomonacide, the results of treatment of vaginal trichomoniasis were poor (Willscox 1961). In 1958 the trichomonacidal effect of metronidazole was found to be excellent in both *in vivo* and *in vitro* experiments (Cosar and Joulou 1959) and so were the results of the first reported clinical trial (Durel *et al.* 1959). Since then several authors have reported cure rates of 90–100 per cent (Table 1).

In 1962 Svendsen from this department reported a cure rate of 97 per cent (74/76). In our daily routine during recent years we have had the impression that the therapeutic effect of the drug is not as good as previously. We have therefore performed a new clinical trial with metronidazole.

### *Material and Method*

The series consists of 120 patients with trichomonal vaginitis seen in the gynaecological out patient clinic at Lillehammer Hospital from February 1967 to December 1967. They were selected according to Svendsen (1962). The diagnosis of trichomoniasis was verified by microscopic examination of wet smear preparations stained with 0.25 per cent Brilliant Cresyl Blue in isotonic saline. Cervical smears for gonococcal cultures were taken in all patients and were positive in 9 cases.

All patients were given 200 mg metronidazole orally three

agreement between cytological and wet smear preparations. Failure to diagnose the presence of flagellates before treatment would exclude the patient from our trial. If the same occurred at the follow-up examination, the cure rate would improve. Thus diagnostic failures cannot explain our bad treatment results.

In comparing our treatment results with those of *Svendsen* (1962) some differences in the composition of the series should be noted. *Svendsen* excluded all pregnant women, and none of his patients had received metronidazole treatment before. In his sample 46 out of 76 patients had established regular sexual partnerships (61 per cent) in our series the percentage was 63. Except for these slight differences the series should be as similar to each other as possible—coming from the same population and treated in the same department. The treatment was identical in both series except that none of *Svendsen's* cases received more than one course. At examination during the first postmenstrual period after treatment *Svendsen* found only two failures and at a second follow-up 3 months later there were 7 with reinfection.

The bad results of treatment in pregnant women cannot explain the difference. If these patients are excluded from our series the cure rate after one course of treatment would be 64 per cent (65/102) and after two courses 78 per cent (79/102). Both of these differences from *Svendsen's* 97 per cent are statistically highly significant ( $p < 0.0005$ ).

Reinfections are probably not more frequent in 1967 than in 1961 the percentage of fixed sexual partnerships being the same.

*Jennison et al.* (1961) and *Scott-Gay* (1964) found no evidence of reduced absorption of the drug in those cases which did not respond to treatment. The manufacturers of the drug have informed us that no changes in the composition of the tablets have been made during these years.

Treatment with higher doses or combined oral and vaginal treatment do not seem to increase the cure rates (*Petryra* and *Deo Lancuz*, 1964; *Watt* 1965; *Sands* 1966).

Already in 1962 *Watt* and *Jennison* concluded that development of resistance against metronidazole was a theoretical pos-

times a day for seven days. The male partners were treated simultaneously when possible. If the first course of therapy failed a second course was given to both partners whenever possible. The patients were re-examined about ten days after the end of the therapy and all received instructions to return later if signs or symptoms of recurrence appeared.

For statistical analysis  $\chi^2$  test was used.

### Results

The results of treatment are shown in Table III. The differences from the results achieved in 1961 are statistically highly significant ( $p < 0.0005$ —after one course of treatment  $\chi^2$  was 31.75 after two  $\chi^2$  was 16.78).

The duration of symptoms did not influence the results of treatment neither did parity, age or the menopause. In patients with a clinical diagnosis of cervicitis the cure rate was the same as in those cases with a normal cervix (73 per cent—35/48). In ten patients previous treatment with metronidazole had been successful, eight of them were cured in this series. Of 13 cases first treated unsuccessfully with nifuratel (Gjønnæss and Aure 1969) 9 were cured with metronidazole.

When the sexual partners could be treated, the cure rate after 2 courses of treatment was 74 per cent (55/74); otherwise it was 75 per cent (33/44). The cure rate in pregnant women was poor, 56 per cent (9/16), but the group is too small to demonstrate a statistically significant difference from non-pregnant women.

### Discussion

A significant decrease in the cure rate of metronidazole treatment of trichomonal vaginitis from 1961 to 1967 is demonstrated.

The verification of the diagnosis of vaginal trichomoniasis has been discussed. Cytological smears, cultures and wet smear preparations all have their advocates. Some investigators have found wet smears to be superior to the other methods (Bernstine and Rakoff 1953; Barnes *et al.* 1957; Bedoya *et al.* 1958). Both Lundström (1960) and Svendsen (1962) found complete

## REFERENCES

- Arnold M., *Thier Umsich.* 23 358 1966  
 Barnes J. Boucwood, A. Halnam E. Livingston, W. and Listers E. *Brit. med. J.* 1, 1160 1957  
 Bedoya, J. M. Rico G. and Rico R. L. *Geburtsh. u. Frauenheilk.* 118 989 1958  
 Bernstein J. B. and Reboff A. E. *Vaginal Infections, Infestation and Disease.* Blackston Co. Inc. 1953  
 Brödlund R. *Nord Med.* 68 1283 1962  
 Coquer C. and Jondou, L. *Ann Inst. Pasteur* 96 238 1959  
 Cronke G. W. *Brit. J. vener. Dis.* 39 258 1963  
 de Cerny I. *Lancet* 1 1042, 1965  
 Dorel M. P. Rotrou V. Siboulet A. and Borell L. *J. C. R. Soc. franç. Gynec.* 29 36 1959  
 Gembert H. L. and Dulles C. D. *Amer. J. Obstet. Gynec.* 89 990 1964  
 Gynnesnes H. and Aure J. C. *Acta obst. et gynec. scandinav.* 48 85 1969  
 Jonsson, R. F. Stenson P. and Warr L. *J. clin. Path.* 14 421 1961  
 Kighley E. E. *Brit. med. J.* 11 93 1962  
 Lundström P. *Acta obst. et gynec. scandinav.* 39 198 1960  
 Nicol C. S. M. C. Fadzom J. A. and Sandret S. L., *Lancet* 1 1100 1960  
 Parker R. T. Thomas W. L. and Jones C. P. *South M. J.* 58 211 1965  
 Perryne, A. J. and Doe Lancrag, J. *Obstet. and Gynec.* 24 499 1964  
 Piri G. *Obstet. and Gynec.* 28 273 1965  
 Peterson W. F. Stenck J. E. and Ryder C. D. *Amer. J. Obstet. Gynec.* 94 343 1956  
 Smids R. *Amer. J. Obstet. Gynec.* 94 350 1966  
 Scott-Gray M. J. *Obstet. Gynec. Brit. Colith.* 71 82, 1964  
 Siendern, E. K. T. *norake legforen* 82 957 1962  
 Teton J. B. and Tredehell N. C. *Obstet. and Gynec.* 21 356 1963  
 Warr L. *Practitioner* 195 813 1965  
 Warr L. and Jonsson R. F. *Brit. med. J.* 1 276 1952  
 Wilcox R. R. *Brit. J. clin. Pract.* 15 233, 1961  
 Odegaard, K. *Nord Med.* 68 1483 1962

Received on June 19 1968

sibility Jennlson *et al* (1961) tested 66 strains of trichomonas for sensitivity to metronidazole seven of them from women who did not respond to treatment. No increased resistance could be demonstrated. Attempts to induce resistance *in vitro* by repeated subcultures have failed (Ødegaard 1962 Watt and Jennlson 1962 Nicol *et al* 1966)

Recently *de Carneri* (1966) presented the first report of *in vitro* development of partially resistant strains of trichomonas. After 3 months culture there was a significant increase of tolerance to metronidazole in one strain. With these strains a threefold increase in drug dosage was needed to effect a cure in subcutaneously infected mice. In 1966 *Arnold* reported a decrease in cure rates at the Universitätsfrauenklinik in Bern from 92.6 per cent in 1960-1961 to 76.4 per cent in 1965-1966. The author suggested that a certain resistance to metronidazole treatment had developed.

The decrease in cure rates in the present investigation is almost identical with the decrease reported by *Arnold* and as no other reason for this can be seen, the authors have come to the same conclusion.

When the cure rates of treatment of trichomonal vaginitis over the period 1960-1967 have decreased significantly from 97 per cent to 73 per cent, this must be due to development of strains of trichomonas vaginalis totally or partially resistant to metronidazole.

## SUMMARY

The results of metronidazole treatment of trichomonal vaginitis in 120 cases (200 mg three times a day for 7 days) are reported. Patients in whom this treatment failed were given a second course of treatment. The cure rate after one course of treatment was 61 per cent after two courses 73 per cent. The results of treatment are compared with those obtained in the same hospital in 1961 (97 per cent). A marked decrease in cure rates is noted. The possible reasons for this decrease are discussed. It is suggested that this decrease must be due to the development of strains of *Trichomonas vaginalis* resistant to metronidazole.

Table I. Hospital Where and Conditions under Which the Procedures Were Performed

	In-Patients	Out-Patients	Total
County Hospital, Hobbs	63	78	139
Central Hospital, Sikeston	234	0	234
Total	297	76	373

Table II. Nature of Procedure in Relation to Patient Age

Nature of Procedure	Age				Total
	20	20-39	40-59	≥ 60	
Curettage for diagnostic purposes	9	105	185	22	321
Curettage for abortion	9	39	4	0	52
Total	18	144	189	22	373

vaginal plexus (Frankenhauser) which is a cluster of ganglia situated in the parametrium around the main trunk of the uterine artery just lateral to and behind the cervix on a level with the internal os. This blocks the afferent impulses from the uterus to the inferior and superior hypogastric plexuses.

A special 15 cm needle is used mounted on an ordinary 20 cc syringe. A guide may be used, but is not necessary.

The needle is inserted into the vaginal fornices. Many authors have reported two injection sites, at 3 and 9 o'clock or at 4 and 8 o'clock. Four or more sites of injection have also been used. In the present series 4 injections were made, at 3 6 9 and 12 o'clock.

As for the depth of injection, the various reports in the literature quote between 1/2 and 2 cm. In the present series the depth was about 1 cm.

Lidocaine (Leostein® Xylocaine®) as well as mepivacaine

From the Department of Surgery (G Toft M.D.) County Hospital, Hobro,  
and the Department of Obstetrics and Gynaecology (P Halkier Sørensen)  
Central Hospital Silkeborg, Denmark

## PARACERVICAL BLOCK

Use In Curettage for Diagnostic Purposes and for Abortion

BY

P I JØRGENSEN

The deposition of a local anaesthetic around the nerves and nervous plexuses of the paracervical tissue for alleviating or eliminating uterine pain in gynaecological procedures by the vaginal route was described as early as 1909 by Wernitz

During the subsequent years the German literature brought several reports of the use of this form of anaesthesia for example for curettage vaginal hysterotomy and hysterectomy (Thaler 1912 Ruge 1912)

In Denmark Hansen in 1948 found paracervical block (p.b.) to be applicable in curettage for legally approved abortions.

In recent years a few American authors have reported good results when using p.b. for curettage for diagnostic purposes as well as for abortion (Mengert and Slate 1960 Connor and Bepko 1964)

But the main use of paracervical anaesthesia is in obstetrics as is apparent from numerous American and now also Scandinavian reports (Gad 1967 Simonsen 1967)

In the present study an attempt has been made to estimate the efficacy and side effects of p.b. in 373 patients who had curettage for diagnostic purposes or for abortion using p.b. anaesthesia.

### *Mechanism of Effect and Technique*

When inserting a p.b. the surgeon tries to deposit the local anaesthetic around the paracervical nerves in particular the utero-



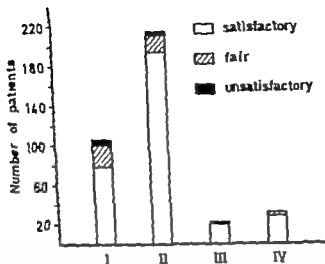


Fig 1 Analgesic effect to curettage for diagnostic purposes and for abortion with and without premedication. Column I Curettage for diagnostic purposes without premedication. Column II Curettage for diagnostic purposes with premedication. Column III Curettage for abortion without premedication. Column IV Curettage for abortion with premedication.

### Efficacy

As premedication about two-thirds of the patients received *Le* injection of pethidine 50 mg, immediately before the insertion of p.b.

The efficacy was estimated after a latent period of 10 minutes and based upon an objective assessment of the patient and her statements concerning freedom from pain during the procedure. By this means, the efficacy could be estimated as

- (1) Satisfactory (no or negligible pain)
- (2) Fair (moderate tolerable pain)
- (3) Unsatisfactory (severe pain)

Fig 1 relates the efficacy to the nature of the procedure and the premedication.

In diagnostic curettage without premedication (106 patients) the effect proved satisfactory in 78 (74 per cent) fair in 23

(Carbocaine<sup>®</sup>) may be used. We used Leostesin-noradrenaline<sup>®</sup> 1 % in most cases 20 ml equally distributed in the 4 sites of injection. As a rule it is recommended that a vasoconstrictor should be used as this is supposed to reduce the risk of side effects and to cause less bleeding and more prolonged analgesia.

As a preliminary the vulva and vagina are washed. Thereafter the cervix is examined with a speculum and grasped with a tenaculum so that the fornices are distinctly visible. The injection is made slowly after it has been established by aspiration that the needle has not entered a vessel.

The time from the insertion of the block until the surgical procedure was started was usually about 10 minutes.

### *Present Material*

The series was collected in the Surgical Department of the County Hospital Hobro in February–October 1966, and in the Gynaecological-Obstetrical Department of the Central Hospital, Silkeborg, during the period November 1966 to April 1967. It includes 373 patients, both out patients and in-patients who had curettage for diagnostic purposes or for abortion with p.b. as analgesia (Table I).

During the period of the study p.b. was the preferred method of anaesthesia for the procedures mentioned. Other methods were used only in cases where p.b. was felt to be less suitable because of anatomical conditions, the patients' apprehension, suspicion of adnexal inflammation or increased risk of haemorrhage (anti-coagulant medication). Apart from these few exceptions (about 10 patients) there was no selection.

Diagnostic curettage was preceded in all cases by dilatation of the cervical canal until it admitted a Hegar 6–7. Some of the patients had a cervical biopsy, the removal of a cervical polyp or curettage of the cervical canal at the same time. In curettage for abortion only a quarter of the patients required dilatation of the cervical canal.

Table II gives the nature of the procedures in relation to the patients' age.

reduce the frequency of this complication which was relatively most common in the age range 40-59 years (17 per cent)

Precordial pain or palpitations occurred in 5 patients, accompanied in 4 by an elevation of blood pressure. In all cases the symptoms soon passed. Two of these patients suffered from chronic bronchial asthma and hypertension.

The 234 patients admitted to the Central Hospital, Stilleborg, had a routine check of their blood pressure immediately before and after insertion of the block and after the procedure was completed.

An elevation of blood pressure ( $\geq 20$  systolic and/or diastolic) was observed in 74 patients (32 per cent) most commonly in the age range 40-59 years. After the procedure the blood pressure had in most cases returned to initial level.

Among 28 patients with headache 22 (79 per cent) showed elevation of blood pressure as compared with 52 (25 per cent) of the remaining 206 patients who did not have headache.

Nausea occurred in 16 patients, only among those who had received premedication.

Among the somewhat later complications, parametrial bleeding, infection, as well as injuries to nerves or organs might have been expected. In the present series only fever and pelvic pain occurred. These complications were assessed only in the in-patients, as out-patient follow-up was not sufficient for this purpose.

Among patients who had a diagnostic curettage 10 (4 per cent) were febrile for a day or two after the procedure and 5 also had pelvic pain.

After curettage for abortion 11 (21 per cent) had a mild elevation of temperature for the first few days. This group includes 8 patients admitted for incomplete abortion, 6 of whom had fever on admission. The last 3 patients were having legally induced abortion, and none of them suffered pain after the procedure.

In all 71 cases the fever had subsided in a few days. Gynaecological examination on discharge did not disclose any changes to explain the febrile reaction. In this connection there is reason to mention a 45-year-old patient who had curettage without any immediate complications and who was discharged 4 days later after gynaecological examination had shown no abnormality. She

(22 per cent) and unsatisfactory in 5 (4 per cent) 214 patients had received premedication for the same procedure. The effect was satisfactory in 194 (91 per cent) fair in 16 (7 per cent) and unsatisfactory in 4 (2 per cent). One patient was excluded, because the insertion of p b had to be interrupted because of side effects (see Side Effects).

Without premedication the effect in curettage for abortion was found to be satisfactory in 20 patients unsatisfactory in one. With premedication it was satisfactory in 29 and fair in 2. In no case did dilatation of the cervical canal prior to curettage for abortion elicit pain.

### *Side Effects*

Simultaneously with the insertion of p b some patients exhibited side effects in the form of shock like states headache precordial pain or palpitations hypertension or nausea.

After the injection of 18 ml of the local anaesthetic a 73-year old patient turned pale complaining of precordial pain as well as malaise. At the same time her blood pressure fell from 170/100 to 70/40. The pulse became soft, but remained regular around 60. No changes in consciousness occurred. After she had been placed in the Trendelenburg position and had received oxygen and dextran the condition soon improved. An emergency ECG as well as an ECG on the following day failed to show any signs of acute changes. The procedure had to be given up and the patient is not included in the assessment of the efficacy of this method.

A quarter of an hour after the insertion of the block a 52-year-old patient who was having a diagnostic curettage complained of malaise. Her blood pressure fell from 150/90 to 90/40. The pulse was unchanged, full around 60. The malaise soon disappeared after she had been placed in the Trendelenburg position and given oxygen and the procedure was carried through.

Forty-four patients (12 per cent) complained of headache during or immediately after the application of the block. In most cases the headache affected the back of the head and usually disappeared in a few minutes. The use of premedication did not

signs of incipient inflammation. There was no case of parametrial haematoma as described, one case each, by *Derrits*, (1964) and *Page* (1961) after p.b. used during delivery.

P.b. requires a minimum of instruments and is relatively easy to insert. An anaesthetist is not needed. In departments having no full-time anaesthetist, in the consulting room, and for patients who do not want general anaesthesia, the method must be considered valuable. In the treatment of patients admitted as emergency cases with profuse bleeding, p.b. has the additional advantage that the patients need not necessarily be fasting and that immediately after the insertion of the block the haemorrhage often decreases appreciably presumably an effect of the noradrenaline.

Its drawbacks are the side effects mentioned above and the inability to check the findings by bimanual examination on a fully relaxed patient.

The only absolute contra indication is allergy to local anaesthetics. Owing to a possibly increased risk of side effects, some caution should be displayed in using the method in patients with hypertension, serious cardiac disease and parametritis, as well as in patients with an increased risk of bleeding.

To meet possible severe side effects, injectable barbiturates as well as apparatus and drugs for resuscitation should always be at hand.

## SUMMARY

Paracervical block was used for 373 patients having curettage for diagnostic purposes and for abortion.

With pethidine as premedication, a satisfactory analgesic effect was observed in 90 per cent of the patients.

A 73-year-old patient developed signs of shock during the insertion of the block. 13 per cent exhibited side effects in the form of headache or mild cardio-vascular complaints, usually accompanied by elevation of blood pressure. There were no late complications that could be definitely ascribed to the block.

Summing up it may be said that the method as used in the

was re-admitted 10 days later with fever and pelvic pain. Laparotomy revealed a large right tubo-ovarian abscess.

On the day after the procedure one patient developed a generalized skin rash which responded to 2 days antiallergic therapy (Antistina® tablets)

No patient complained of bladder or bowel disturbances or of neurological symptoms.

### Discussion

The object of this study was to assess the applicability of p.b. in curettage of the uterus.

The analgesic effect with pethidine given simultaneously as premedication was satisfactory in more than 90 per cent of the patients. Others have obtained similar results. Hansen, in 1948, found good analgesia in 89 out of 90 patients who had dilatation of the cervical canal and curettage for abortion after insertion of p.b. Mengert and Slate 1960 used p.b. in 377 out-patients who had dilation of the cervical canal and curettage for diagnostic purposes. No premedication was given. The analgesic effect was good in about 90 % of the patients. Connor and Bepko 1964 treated 605 patients by curettage for abortion, using paracervical-pudendal block after barbiturate premedication. The analgesic effect was satisfactory in all cases but one.

The incidence of side effects immediately after the injection was relatively high. Thus 44 patients (12 per cent) developed headache and 5 cardiac symptoms. The fact that in most cases these complaints were accompanied by elevation of blood pressure renders it likely that noradrenaline was responsible. Apart from one of the two patients who exhibited a shock like state, however there were no serious side effects during the insertion of p.b. It may be mentioned that in the literature on p.b. no similar side effects have been reported except for a single case of "hypotensive reaction" for which only a few details are given (Mengert and Slate 1960).

None of the late complications was serious. Nearly all were

signs of incipient inflammation. There was no case of parametrial haematoma as described, one case each, by *Dennis*, (1964) and *Page* (1961) after p.b. used during delivery.

P.b. requires a minimum of instruments and is relatively easy to insert. An anaesthetist is not needed. In departments having no full-time anaesthetist, in the consulting room, and for patients who do not want general anaesthesia, the method must be considered valuable. In the treatment of patients admitted as emergency cases with profuse bleeding, p.b. has the additional advantage that the patients need not necessarily be fasting and that immediately after the insertion of the block the haemorrhage often decreases appreciably presumably an effect of the noradrenaline.

Its drawbacks are the side effects mentioned above and the inability to check the findings by bimanual examination on a fully relaxed patient.

The only absolute contra-indication is allergy to local anaesthetics. Owing to a possibly increased risk of side effects, some caution should be displayed in using the method in patients with hypertension, serious cardiac disease, and parametritis, as well as in patients with an increased risk of bleeding.

To meet possible severe side effects injectable barbiturates as well as apparatus and drugs for resuscitation should always be at hand.

## SUMMARY

Paracervical block was used for 373 patients having curettage for diagnostic purposes and for abortion.

With pethidine as premedication, a satisfactory analgesic effect was observed in 90 per cent of the patients.

A 73-year-old patient developed signs of shock during the insertion of the block. 13 per cent exhibited side effects in the form of headache or mild cardio-vascular complaints, usually accompanied by elevation of blood pressure. There were no late complications that could be definitely ascribed to the block.

Summing up it may be said that the method as used in the

present series proved simple, cheap and sufficiently effective. However, side effects were relatively common but generally not serious.

#### REFERENCES

- Connar E J and Bepko F J *Am. J. Obst. Gyn.* 89 822, 1964  
Dennis K J *J. Obst. Gyn. Brit. Cmwth.* 71 797 1964  
Gad C *Acta obst. gynec. scand.* 46 7 1967  
Hansen J L *Ugeskr. Læg.* 110 728 1948  
McGert W F and Slate W G *Am. J. Obst. Gyn.* 79 727 1960  
Page E P, Hamm M L and Chappell C C *Am. J. Obst. Gyn.* 81 1094 1961  
Ruge E *Zentralbl. Gynäk.* 36 561 1912  
Simonsen M *Ugeskr. Læg.* 35 1106, 1967  
Thaler H *Zentralbl. Gynäk.* 36 63 1912  
Wernitz *Zentralbl. Gynäk.* 33 1083 1909

Received on June 2, 1968







## MEASUREMENT OF HUMAN UTERINE CERVICAL BLOOD FLOW BY LOCAL HYDROGEN GAS CLEARANCE

BY

INGE KLINGENBERG AND KNUT AUKLAND

Present knowledge of local blood flow in the human myometrium is based on relatively few observations. Total uterine blood flow has been measured by electromagnetic flowmeter (Assali *et al.* 1960) and by  $N_2O$ -technique (Assali *et al.* 1953 Mercalfe *et al.* 1955) but no information on local blood flow in the myometrium can be obtained by these methods. A relative measure of local myometrial blood flow may be obtained by heat conductivity (Prill 1959) or local clearance of  $Na^{24}$  (McCall and Sopp 1951) but neither method gives an absolute flow measurement. Theoretically local clearance of Xenon<sup>133</sup> injected into the myometrium can be interpreted in terms of absolute flow per gram of tissue and measurements have been described both for isthmus and cervix in pregnant and non pregnant women (Munck *et al.* 1964 Lysgaard and Lefèvre 1965).

This article presents data on local blood flow in the fibromuscular tissue of the cervix in humans measured by the hydrogen desaturation technique (Aukland *et al.* 1964). The criteria for acceptable measurements are discussed on the basis of studies on the reproducibility of subsequent measurements in a given area, and on comparison of simultaneous measurements in different areas of the cervix.



time will therefore give a linear desaturation curve with a slope determined by  $k$ . The numerical value of  $k$  is conveniently determined from the half time,  $T_{\frac{1}{2}}$  which is the time required for tissue concentration to be reduced to half its numerical value. Equation (4) may thus be written

$$\ln C_1 = \ln C_0 - kt$$

$$\text{or} \quad k = \frac{\ln C_0 - \ln C_1}{t} \quad (5)$$

For 50 % reduction of concentration

$$k = \frac{\ln 100 - \ln 50}{T_{\frac{1}{2}}} = \frac{0.693}{T_{\frac{1}{2}}}$$

$$\text{Since } k = \frac{F}{Wt}$$

$$\frac{F}{W} = \frac{0.693}{T_{\frac{1}{2}}} \quad (6)$$

As the tissue/blood partition coefficient is close to unity in such different tissues as kidney (Aukland et al. 1964) and brain (Fieschi et al. 1964) the myometrium/blood partition coefficient is also assumed to be 1.00. Therefore

$$\frac{F}{W} = \frac{0.693}{T_{\frac{1}{2}}} \quad (7)$$

If  $T_{\frac{1}{2}}$  is measured in minutes local flow  $F/W$  is obtained in ml/min per gram tissue

Fig. 1 illustrates schematically the polarographic method for measuring hydrogen concentration. When a platinum electrode placed in saline is connected to a silver/silver chloride reference electrode and polarized by a dry cell at a potential of +0.3 volts relative to the reference only minimal current is produced in the circuit. If hydrogen gas is then added to the solution, hydrogen

### Methods

Local myometrial blood flow was measured by polarographic recording of hydrogen gas desaturation (Aukland *et al.*, 1964). The method is based on the theory of Kery (1951) for gas exchange between blood and tissue. According to Fick's principle, the amount of test substance brought to an organ by arterial blood is equal to the sum of the amount of the test substance metabolized the amount accumulated in the organ and the amount removed by venous blood. The present test substance, hydrogen gas is not metabolized. It is assumed that tissue blood volume is unchanged during the investigation period and that blood flow ( $F$ ) is constant.

If the concentration of hydrogen in arterial blood is  $C_a$ , in venous blood  $C_v$  and the hydrogen concentration change  $dC_t$  in the tissue volume  $W$  during the time  $dt$ , the Fick principle gives the equation

$$dC_t W = FC_a dt - FC_v dt \quad (1)$$

Because of the high diffusibility of hydrogen gas, one may assume diffusion equilibrium between tissue and venous blood during the saturation and desaturation period. If the tissue/blood partition coefficient for hydrogen is  $\lambda = C_t/C_v$  equation (1) becomes

$$dC_t W = F(C_a - C_t/\lambda) dt \quad (2)$$

When arterial hydrogen concentration is suddenly lowered to zero ( $C_a = 0$ ) equation (2) yields

$$dC_t = \frac{C_t F}{W\lambda} dt \quad (3)$$

Integration between the limits  $t = 0$  and  $t = t$  yields

$$C_t = C_{t0} e^{-kt} \quad (4)$$

where  $C_{t0}$  is the initial tissue concentration and  $k = \frac{F}{W\lambda}$  showing that tissue concentration falls as an exponential function of time. Hydrogen concentration plotted on a logarithmic scale against

time will therefore give a linear desaturation curve, with a slope determined by  $k$ . The numerical value of  $k$  is conveniently determined from the half time,  $T_{1/2}$  which is the time required for tissue concentration to be reduced to half its numerical value. Equation (4) may thus be written

$$\ln C_t = \ln C_{\infty} - kt$$

$$\text{or} \quad k = \frac{\ln C_{\infty} - \ln C_t}{t} \quad (5)$$

For 50 % reduction of concentration

$$k = \frac{\ln 100 - \ln 50}{T_{1/2}} = \frac{0.693}{T_{1/2}}$$

$$\text{Since } k = \frac{F}{Wt}$$

$$\frac{F}{W} = \frac{0.693}{T_{1/2}} \quad (6)$$

As the tissue/blood partition coefficient is close to unity in such different tissues as kidney (Aukland *et al.* 1964) and brain (Fieschi *et al.* 1964) the myometrium/blood partition coefficient is also assumed to be 1.00. Therefore

$$\frac{F}{W} = \frac{0.693}{T_{1/2}} \quad (7)$$

If  $T_{1/2}$  is measured in minutes local flow  $F/W$  is obtained in ml/min per gram tissue

Fig. 1 illustrates schematically the polarographic method for measuring hydrogen concentration. When a platinum electrode placed in saline is connected to a silver/silver chloride reference electrode and polarized by a dry cell at a potential of +0.3 volts relative to the reference only minimal current is produced in the circuit. If hydrogen gas is then added to the solution, hydrogen

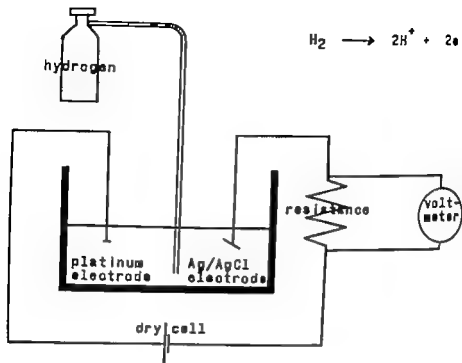


Fig. 1 Principle of hydrogen polarography

molecules will be oxidized at the platinum surface, thereby producing a current proportional to the concentration of dissolved hydrogen gas.

Measurements in the cervical fibromuscular tissue were performed with needle-shaped electrodes made of 0.8 mm thick platinum wire. The 20–30 mm long electrodes were insulated with lacquer except for the 1.5 mm-long pointed tip, which was left bare. The silver/silver chloride electrode was formed as a  $2.6 \times 3.3$  cm sheet and fixed by adhesive tape to the skin prepared with electrolyte ointment. The electrode current was measured with a Keithley 150 A microvolt ammeter and recorded on a Honeywell Brown electronic recorder (Fig. 2). With a 6-way switch, six electrode circuits could be read alternately without changing the polarizing potential (Aukland *et al.* 1967).

The electrically insulated patient lay in bed during the investigation. No anaesthetics or other drugs were used. With a speculum in the vagina, the cervix was grasped with volsella and two or



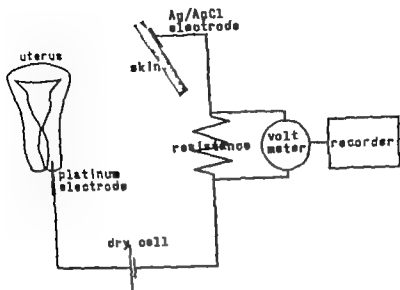


Fig 2 Hydrogen polarography applied to uterine cervix blood flow measurement

three platinum electrodes were inserted into the cervix parallel to the cervical canal. The depth of electrode insertion was measured in each case, but the exact position relative to the cervical canal was difficult to assess. By probing the cervical canal with a sound, the electrode tip was sometimes found to have penetrated to the mucosa or submucosa of the canal. Measurement from such electrode positions were discarded. After electrode insertion, the volsella and speculum were removed.

Hydrogen gas was added to the respiratory air by open mask, giving an alveolar hydrogen concentration of 5-15 volume per cent. When the uterine cervical tissue had reached a steady concentration, indicated by the electrode current levelling off the administration of hydrogen was stopped and the patient asked to hyperventilate forcefully for approximately 20 seconds. Hydrogen was thereby rapidly removed from the alveoli and arterial blood, and cleared from the cervical tissue at a rate proportional to local blood flow. The current produced by the platinum electrode

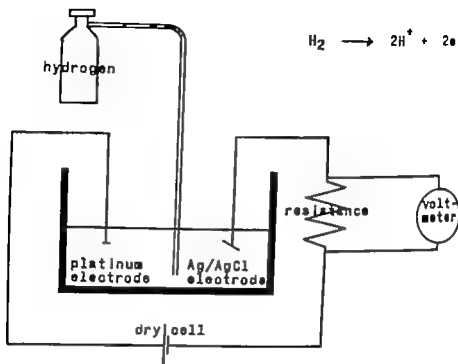


Fig. 1 Principle of hydrogen polarography

molecules will be oxidized at the platinum surface thereby producing a current proportional to the concentration of dissolved hydrogen gas.

Measurements in the cervical fibromuscular tissue were performed with needle-shaped electrodes made of 0.8 mm thick platinum wire. The 20–30 mm long electrodes were insulated with lacquer except for the 1.5 mm long pointed tip, which was left bare. The silver/silver chloride electrode was formed as a  $2.6 \times 3.3$  cm sheet and fixed by adhesive tape to the skin prepared with electrolyte ointment. The electrode current was measured with a Keithley 150 A microvolt ammeter and recorded on a Honeywell Brown electronic recorder (Fig. 2). With a 6-way switch six electrode circuits could be read alternately without changing the polarizing potential (Aukland *et al.* 1967).

The electrically insulated patient lay in bed during the investigation. No anaesthetics or other drugs were used. With a speculum in the vagina the cervix was grasped with volsella and two or

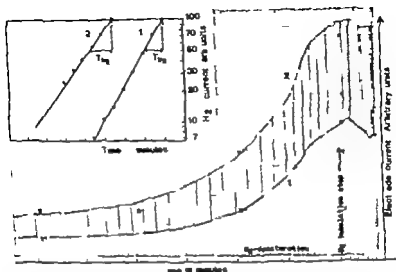


Fig. 3 Hydrogen desaturation curves recorded simultaneously from two different electrodes in the cervix of 52-year-old woman with irregular uterine bleeding. Inset semilogarithmic plot of the desaturation curves. Curve 1 monoexponential, curve 2, multiexponential. Straight lines show the slopes used for flow calculation.

3. Each tissue compartment is represented in the summation curve according to its weight (Ingvar and Lassen 1962)

The second assumption was satisfied by sufficiently long equilibration time but the first and third assumptions may not be valid, as discussed by Auckland *et al* (1967) and may cause too high a flow estimate.

To some extent this conclusion can be evaluated empirically from the present data. Monoexponential curves were obtained in 109 measurements in 45 patients, and multiexponential curves in 107 measurements in 44 patients (in some patients, both mono- and multiexponential curves were obtained). The mean age in the monoexponential group was 43.3 and in the multiexponential 44.1. Table 1 shows that the two groups were also comparable with respect to diagnosis. Fig. 4 indicates that flow distribution was similar in the two groups although flow values from mono-

gave a continuous indication of the hydrogen concentration in the tissue.

The first measurement was undertaken 20–30 minutes after insertion of the electrodes. The second measurement was performed 20–30 minutes later and in some cases blood flow was measured up to 4½ hours after electrode insertion.

### *Results and Discussion*

A total of 216 desaturation curves were recorded from 116 different electrode sites in 60 patients aged 20 to 71 years the majority being between 30 and 55 years. A further thirteen curves were excluded for technical reasons.

Original desaturation curves recorded simultaneously from two electrodes placed in the anterior lip of the cervix of a 52 year old woman suffering from irregular bleeding, are reproduced in Fig 3 lower right part. A semi logarithmic plot of the same curves is given in the upper left part of the same figure. One of the electrodes showed a good monoexponential desaturation as predicted theoretically for homogenous blood flow in the small tissue volume surrounding the electrode tip (Cf equation 4) The other curve shown in Fig 3 clearly deviates from monoexponential desaturation and this was a relatively frequent finding. To evaluate the significance of the different curve shapes, all curves were divided arbitrarily into monoexponential and multiexponential; the criterion for monoexponential being a practically linear semi logarithmic plot down to at least 80 per cent desaturation. It is obvious that this criterion leaves considerable margin for subjective judgment but we were unable to define more strict criteria for routine use.

For multiexponential curves blood flow was calculated from the initial slope of the desaturation curve. This procedure is theoretically correct, provided that

1. The multiexponential desaturation is due to nonhomogeneous capillary blood flow and accordingly different desaturation rates in the tissue surrounding the electrode
2. All tissue compartments are in parallel and completely equilibrated with arterial blood at the start of desaturation, and

ml/min 100g

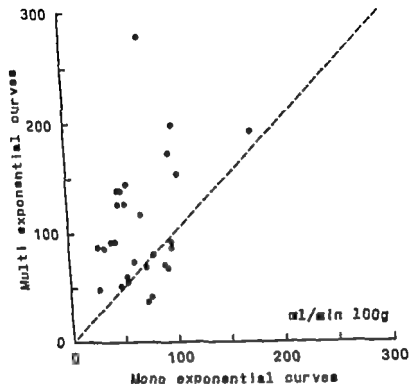


Fig 5 Comparison between simultaneous measurements with two electrodes giving mono- and multiexponential curves respectively

multiexponential curves gives too high an assessment of absolute flow per gram of tissue

Simultaneous measurements at different electrode sites showed considerable scatter among monoexponential curves, as is evident from Fig 6. Multiexponential curves showed even greater differences. The standard deviation for monoexponential curves, judged from simultaneous measurements, was 20.3 per cent of their mean, for simultaneous multiexponentials 30.4 per cent, and for mono-multiexponential pairs 42.3 per cent. Even with mono-

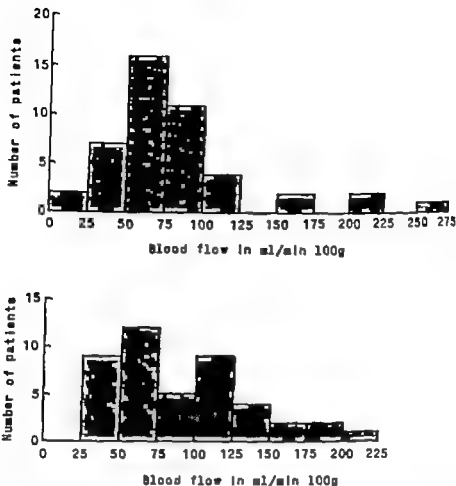


Fig. 4 Distribution of blood flow in 60 patients. Mean flow for each patient obtained from 1-3 measurements, each with 1-3 electrodes. Upper part: Monoexponential curves (43 patients) Lower part: Multiexponential curves (44 patients)

exponential curves showed a somewhat closer grouping around the mean.

As is evident from Fig 5 where simultaneous measurements from different electrode sites are compared multiexponential curves gave on average higher flow rates than monoexponential 109 and 75 ml/min 100 g respectively. As the monoexponential curves do not present theoretical problems in calculating blood flow it seems reasonable to conclude that the initial slope of

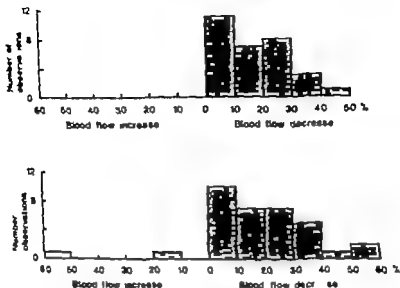


Fig 7 Percentage change in blood flow from first to second measurement at the same electrode site. Upper part Monoexponential curves. Lower part Multiexponential curves.

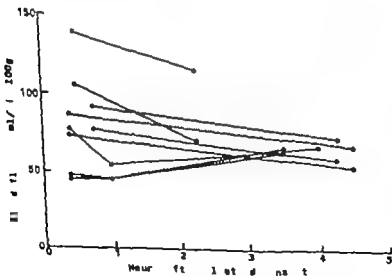


Fig 8 Blood flow measurements at different times after electrode insertion.

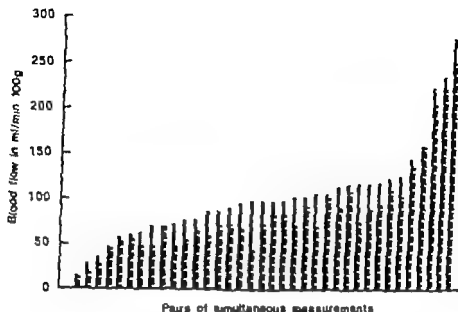


Fig. 6. Comparison between pairs of monoexponential curves obtained in simultaneous measurements.

exponential curves the scatter is about twice as great as for measurements in the myocardium of dogs, using the same technique (Aukland *et al.*, 1967). This suggests that to large extent the variations reflect real flow differences between various areas of the cervix. No consistent flow differences were observed between anterior or posterior lip, nor between varying depths of electrode implantation in the range of 5–20 mm. It seems likely however that the mixture of muscle and connective tissue in the human cervix may be the cause of more heterogenous blood flow than was observed in the myocardium of dogs, and that this is reflected both in the frequent occurrence of multiexponential desaturation curves and the considerable scatter between results from different electrode positions. Scar tissue and other pathological changes might also add to variability.

The conclusion that the scatter between different electrode sites is mainly due to local differences and not to methodological errors, is also supported by the good reproducibility with repeated measurements from electrodes in the same place. However flow estimated by the first measurement, was generally higher than



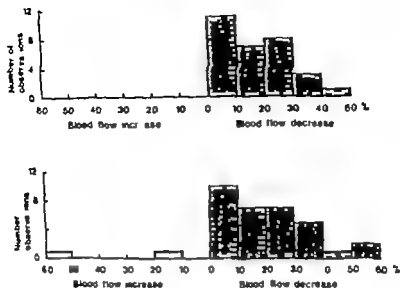


Fig 7 Percentage change in blood flow from first to second measurement at the same electrode site. Upper part Monoexponential curves. Lower part Multiexponential curves.

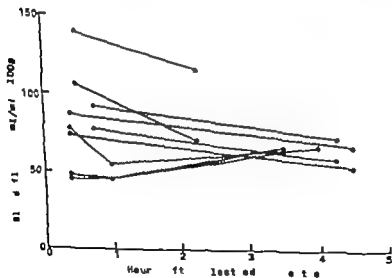


Fig 8 Blood flow measurements at different times after electrode insertion.

that in the second measurement 20–30 minutes later. As is evident from Fig. 7 which shows percentage changes from first to second measurements this tendency was clear both from mono- and multiexponential curves although the scatter was greater in the latter. This observation suggests that multiexponential curves may also show relative changes in local blood flow but should probably not be interpreted in terms of absolute flow. Repeated measurements up to about 4 hours after electrode insertion (Fig. 8) showed a mean decrease of 9 per cent from the first measurement, suggesting that no consistent flow changes take place after the first hour of electrode insertion. It seems probable that the manipulation of the cervix during insertion of the electrodes might induce transitory changes in blood flow and that the second measurement is more representative of physiological blood flow.

Monoexponential curves were obtained in 45 of 60 patients, and the following discussion on absolute blood flow includes only these measurements (Table I). The mean flow rate for individual patients calculated from one to three measurements each with 1–3 electrodes gave flow values ranging from 13 to 264 ml/min 100 g. Most measurements were between 50 and 100 ml/min 100 g, and had a mean of 83 ml/min 100 g. Five patients had cervical blood flow greater than 150 ml/min 100 g. Three of these had uterine myomata and their blood flow was 153, 212 and 221

Table I *Diagnosis and Types of Desaturation Curves*

Diagnosis	Number of Patients		
	Total	Monoexp. Curves	Multiexp. Curves
Pregnancy 6–12 weeks of gestation	8	8	6
Metrorrhagia	20	13	17
Uterine myomata	12	10	8
Carcinoma of cervix or corpus	5	4	1
Uterine prolapse	4	2	2
Vaginal prolapse	4	3	4
Other	7	5	11
	60	45	44

Table II. Blood Flow Obtained from Monoexponential Curves

Diagnosis	Number of Patients	Mean Age (Years)	Mean Blood Flow $\pm$ S.E. ml/min 100 g
Pregnancy 6-12 weeks of gestation	8	33.7	69.4 $\pm$ 6
Metrorrhagia	13	41.1	73.4 $\pm$ 5.8
Uterine myomata	10	47.4	116.6 $\pm$ 19.2
Carcinoma of cervix or corpus	4	49.0	66.8 $\pm$ 9.0
Uterine prolapse	2	57.0	25.5
Vaginal prolapse	3	53.7	78.0
Other	5	40.2	117.6
Total	45	43.3	82.9 $\pm$ 7.9

ml/min 100 g respectively. A 20-year-old girl with a normal genital tract had a mean blood flow value of 264 ml/min 100 g, and in one patient admitted for uterine bleeding where a curettage had been performed the day before measurement, blood flow was found to be 173 ml/min 100 g. The lowest blood flow (13 ml/min 100 g) was obtained in a patient aged 71 who had a vault prolapse following a subtotal hysterectomy. Cervical blood flow in early pregnancy was found to be about the same as in non-pregnant women. Two older patients with uterine prolapse both had low cervical blood flow (23 and 28 ml/min 100 g). Patients with uterine myomata had significantly higher cervical blood flow than the rest of the patients ( $p=0.05$ ) (Table II). Otherwise as shown in Table II the wide variations were not clearly correlated to diagnoses. Furthermore no correlation was observed between cervical blood flow and age or parity.

Considerably lower values have been obtained by external recording of clearance of Xenon<sup>133</sup> injected into the myometrium. In three non-pregnant women Munck *et al.* (1964) found cervical blood flow ranged from 9 to 14 ml/min 100 g, and isthmus flow between 15 and 35 ml/min 100 g. They observed values between 4 and 37 ml/min 100 g in the body of pregnant uteri. Lysgaard and Lefèvre (1965) using the same technique reported blood flow varying between 2 and 32 ml/min 100 g in the myometrium during pregnancy. McCall and Sopp (1951) found clearance

that in the second measurement 20–30 minutes later. As is evident from Fig. 7 which shows percentage changes from first to second measurements this tendency was clear both from mono- and multiexponential curves although the scatter was greater in the latter. This observation suggests that multiexponential curves may also show relative changes in local blood flow but should probably not be interpreted in terms of absolute flow. Repeated measurements up to about 4 hours after electrode insertion (Fig. 8) showed a mean decrease of 9 per cent from the first measurement suggesting that no consistent flow changes take place after the first hour of electrode insertion. It seems probable that the manipulation of the cervix during insertion of the electrodes might induce transitory changes in blood flow and that the second measurement is more representative of physiological blood flow.

Monoexponential curves were obtained in 45 of 60 patients, and the following discussion on absolute blood flow includes only these measurements (Table 1). The mean flow rate for individual patients calculated from one to three measurements, each with 1–3 electrodes gave flow values ranging from 13 to 264 ml/min 100 g. Most measurements were between 50 and 100 ml/min 100 g, and had a mean of 83 ml/min 100 g. Five patients had cervical blood flow greater than 150 ml/min 100 g. Three of these had uterine myomata and their blood flow was 153, 212 and 221

Table 1 *Diagnosis and Types of Desaturation Curves*

Diagnosis	Number of Patients		
	Total	Monoexp. Curves	Multilexp. Curves
Pregnancy 6–12 weeks of gestation	8	8	6
Metrorrhagia	20	13	17
Uterine myomata	12	10	8
Carcinoma of cervix or corpus	5	4	1
Uterine prolapse	4	2	2
Vaginal prolapse	4	3	4
Other	7	5	6
	60	45	44

Table II. Blood Flow Obtained from Monoexponential Curves

Diagnosis	Number of Patients	Mean Age (Years)	Mean Blood Flow $\pm$ S.E. ml/min 100 g
Pregnancy 6-12 weeks of gestation	8	33.7	69.4 $\pm$ 6
Metrorrhagia	13	41.1	73.4 $\pm$ 5.8
Uterine myomata	10	47.4	116.6 $\pm$ 19.2
Carcinoma of cervix or corpus	4	49.0	68.8 $\pm$ 9.0
Uterine prolapse	2	57.0	25.5
Vaginal prolapse	3	53.7	78.0
Other	5	40.2	117.6
Total	45	43.3	82.9 $\pm$ 7.9

ml/min 100 g respectively. A 20-year-old girl with a normal genital tract had a mean blood flow value of 264 ml/min 100 g and in one patient admitted for uterine bleeding where a curettage had been performed the day before measurement, blood flow was found to be 173 ml/min 100 g. The lowest blood flow (13 ml/min 100 g) was obtained in a patient aged 71 who had a vault prolapse following a subtotal hysterectomy. Cervical blood flow in early pregnancy was found to be about the same as in non-pregnant women. Two older patients with uterine prolapse both had low cervical blood flow (23 and 28 ml/min 100 g). Patients with uterine myomata had significantly higher cervical blood flow than the rest of the patients ( $p=0.05$ ) (Table II). Otherwise as shown in Table II the wide variations were not clearly correlated to diagnoses. Furthermore, no correlation was observed between cervical blood flow and age or parity.

Considerably lower values have been obtained by external recording of clearance of Xenon<sup>133</sup> injected into the myometrium. In three non-pregnant women *Munck et al.* (1964) found cervical blood flow ranged from 9 to 14 ml/min 100 g, and isthmus flow between 15 and 35 ml/min 100 g. They observed values between 4 and 32 ml/min 100 g in the body of pregnant uteri. *Lysgaard and Lefèvre* (1965) using the same technique, reported blood flow varying between 2 and 32 ml/min 100 g in the myometrium during pregnancy. *McCall and Sopp* (1951) found clearance

constants for  $\text{Na}^{24}$  of 0.04 to 0.65  $\text{min}^{-1}$  but since  $\text{Na}^{24}$  is not freely diffusible across cell membranes sodium clearance cannot be interpreted in terms of absolute blood flow

The cervical blood flow obtained by the present technique was indeed higher than expected. It is difficult to understand why the cervical tissue should have a blood flow as high as myocardium (Gregg, 1963) or brain (Kety 1960). We believe however that errors which could result in falsely high values such as too short saturation time and multiexponential desaturation curves, have been excluded. The tissue/blood partition coefficient remains to be determined for the myometrium, but it seems unlikely that this could alter the calculated flow significantly.

The main advantages of the hydrogen desaturation method are that blood flow can be measured simultaneously in different places and that measurements can be repeated with high reproducibility in a given area. The measurements are easily carried out, are without complications and involve only slight discomfort to the patient.

## SUMMARY

Blood flow was measured in the human uterine cervix by local clearance of hydrogen gas recorded from needle-shaped platinum electrodes inserted into the cervical tissue. A description is given of the theoretical basis for the method and the practical performance of the measurements.

Repeated measurements from a given electrode site showed good reproducibility whereas simultaneous measurements from different electrode sites showed considerable scatter (SD 20 per cent) probably reflecting local differences in blood flow. Acceptable absolute flow measurements were obtained in 45 out of a heterogeneous series of 60 patients and gave a mean flow of 83  $\text{ml/min } 100 \text{ g}$ . The average cervical blood flow in eight women in early pregnancy was 69  $\text{ml/min } 100 \text{ g}$ . This was not significantly different from that in the remaining cases in the series. The highest average flow was found in patients with uterine myomata.

The main advantages of the method are that local blood flow can be measured a) repeatedly at the same site, and b) at different sites simultaneously

Supported by a grant from Norsk Forening til Krefstens Bekjempelse.

# REFERENCES

- Amat, N S, Douglas R. A. jr, Beard W. W., Nicholson D. B. and Strydom R. *Am. J. Obstet. Gynec.* 66: 248, 1953
- Amat, N S, Rasmussen L. and Pettersen T., *Am. J. Obstet. Gynec.* 79: 86, 1960
- Auland K., Bower B. F. and Berliner R. W. *Circulat. Res.* 14: 164, 1964
- Auland K., Køl F., Kjeldsen J. and Semb G., *Acta physiol. scandinav.* 70: 99, 1967
- Fieschi C., Borzao L., Agnoli A. and Kety S. S. *Soc. It. Biol. Sper.* 40: 1505, 1964
- Gregg, D. E. and Fisher L. C. Blood supply to the heart. In *Handbook of Physiology* Sec. 2, Vol. II, Circulation. Ed. by Hamilton, W. F. and Dow P. Am. Physiol. Soc. Washington, D. C. 1963, 1517
- Ingvar D. H. and Larsson N. A. *Acta physiol. scandinav.* 54: 325, 1962
- Kety S. S. *Pharmacol. Rev.* 3, 1: 1951
- The cerebral circulation. In *Handbook of Physiology* Sec. 1, Vol. III, Neurophysiology. Ed. by Field, J., Magnus, H. W. and Hall, V. E. Am. Physiol. Soc. Washington, D. C. 1960, 1751
- Lysgaard H. and Lefevre H. *Acta obst. et gynec. scandinav.* 44: 401, 1965
- McCall M. L. and Sopp T. *Ann. J. M. Sci.* 221: 113, 1951
- Mercall J., Ramsey S. L., Ramsey L. H., Reid D. E. and Burnell C. S., *J. Clin. Invest.* 34: 1632, 1955
- Musch O., Lysgaard H., Pontander G., Lefevre H. and Larsson N. A. *Lancet* I: 1421, 1964
- Phil, H. J. *Ztschr. Geburtsh. Gynäk.* 152, 69: 1959
- Doberm.* 152, 180, 1959

## MEASUREMENT OF BLOOD FLOW IN HUMAN MYOMETRIUM BY LOCAL HYDROGEN CLEARANCE

BY

INGE KLINGENBERG

Measurement of blood flow in the human uterine cervix by a hydrogen clearance technique has been reported previously (Klingenberg and Aukland 1969). In order to perform similar measurements in the human myometrium a special instrument was constructed, permitting simultaneous measurements from two different areas of the myometrium. The instrument was tested in 27 unanaesthetized nonpregnant women and gave technically satisfactory measurements in all except 5 cases. The observed myometrial blood flow was compared to simultaneous measurements in cervical tissue.

### *Methods*

A 3 cm long, needle-shaped, lacquer insulated platinum wire with a diameter 0.8 mm is soldered to a teflon-insulated copper wire and fixed in place in a steel tube with epoxyresin (Araldite Ciba) (Fig. 1). The steel tube has an outer diameter of 1.5 mm and a length of 21 cm. The lacquer insulation on the 2.5 mm long free end of the tapered platinum wire is removed. Two such electrode tubes are soldered together side by side up to a point 4.5 cm from the electrode tips. In the resting position the electrode tips are 1.5 cm apart, but can be pressed together and kept in this position by a slide. The other end of the united electrode tubes is soldered to a 2.5 cm long steel cylinder provided with screw threads for a



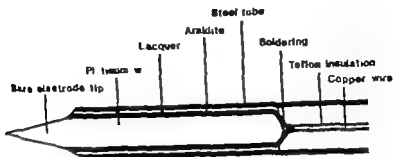


Fig. 1 Mounting of platinum electrode in steel tube.

stop plate, which can be adjusted by means of two nuts. The electrode tubes fit into an introducer made of a steel tube with outer diameter 4 mm. One end of the introducer is closed and rounded, and 3 cm from this blunt end there are two slits for the electrodes (Fig. 2, upper part). The electrodes are inserted into the introducer with the electrodetips pressed together by the slide. During insertion, the slide is retracted by the open end of the introducer allowing the electrodetips to emerge through the slits in the introducer (Fig. 2, lower part). By means of the stop plate and the nuts, the position of the electrode tips can be regulated relative to the blunt end of the introducer. A globe chain, which fits into a track in the stop plate is soldered to a small tenaculum forceps.

During insertion of the instrument the patient is lying in lithotomy position, electrically insulated. No anaesthetics or other drugs are used. The cervix is exposed with a speculum cleansed and pulled down with the small tenaculum forceps. With the electrodes shielded in the introducer the instrument is introduced through the cervical canal until it touches the fundus of the uterus. The electrode tubes are then pushed forwards so that the myometrium is impaled by the electrodes. The electrodes are kept in this position by the small tenaculum forceps, which is locked tight to the stop plate by the globe chain. In most measurements, the electrode tips were adjusted to project 3–4 mm beyond the end of the introducer. For simultaneous measurements in the myometrium of the fundus and the cervix, the electrodes for

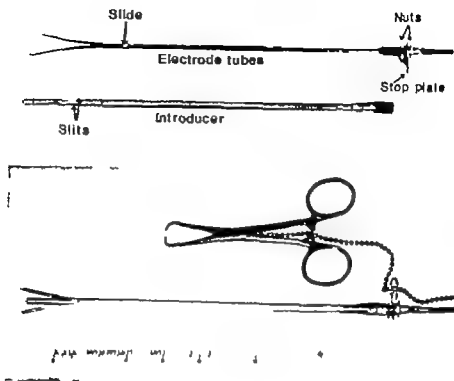


Fig. 2. Instruments for myometrial blood flow measurement.

cervical recordings were inserted before introducing the myometrial instrument. Measurements of hydrogen gas clearance were not started until 20–30 minutes after insertion of the electrodes. The polarographic determination of hydrogen has been described previously (Klingenberg and Aukland 1969) and the same technique was used in this investigation.

### Material

Blood flow measurements in the myometrium were performed in 27 women and simultaneous cervical measurements were obtained in 21 women. The age range was from 20 to 78 years, and the diagnoses are shown in Table I.

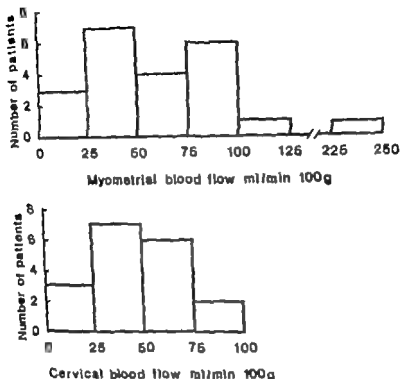


Fig 3 Upper part Distribution of myometrial blood flow in 22 patients. Lower part Distribution of cervical blood flow in 18 patients.

### Results

A total of 56 hydrogen desaturation curves was recorded from the myometrium in 27 patients. Thirty-nine curves in 22 patients were monoexponential to at least 80 per cent desaturation. In 17 patients, monoexponential curves were obtained from both electrodes in the myometrium, and accordingly one of the two desaturation curves was monoexponential in 5 patients. In 2 patients, recordings from one of the electrodes failed for technical reasons, and multieponential curves were obtained from the other electrode. Both myometrial desaturation curves were multieponential in 3 other patients in one of them also after repeated measure-

Table 1 *Average Myometrial and Cervical Blood Flow*

Diagnosis	Number of Patients	Mean Age	Mean Blood Flow ml/min 100 g	
			Myometrium	Cervix
Uterine and vaginal prolapse	5	63	53	32
Metrorrhagia	4	54.5	57	49
Uterine myomata	3	48.5	98	58
Other	5	36.4	50	57

ments had been made with the electrodes in the same position. Since multiexponential curves are difficult to interpret (Klingenberg and Aukland 1969) the results presented in this paper are based solely on monoexponential curves.

The distribution of mean blood flow obtained from 1-2 electrodes in the myometrium of 22 patients is shown in Fig. 3, upper part giving an average of 64.2 ml/min 100 g. In one patient, blood flow was found to be 225 ml/min 100 g, the others had blood flow varying from 22 to 109 ml/min 100 g. As is evident from Table 1 no consistent flow differences were observed between groups of patients with different diagnosis. The high mean flow in the group with uterine myomas is due to high readings (225 ml/min. 100 g) in one patient.

Simultaneous measurements in the myometrium showed good agreement, as illustrated in Fig. 4 where values from two electrodes are placed at random on the abscissa and ordinate respectively.

Cervical measurements in 18 patients (Fig. 3 lower part) showed a flow distribution similar to that in the myometrium. Average flow was 47.2 ml/min 100 g, with a range of 17 to 98 ml/min 100 g. In patients with uterine prolapse cervical flow was found to be relatively low. Otherwise no correlation between cervical flow and age or diagnosis could be demonstrated.

Simultaneous measurements in the myometrium and cervix were performed in 17 patients. As is evident from Fig. 5 the relationship between myometrial and cervical blood flow varied

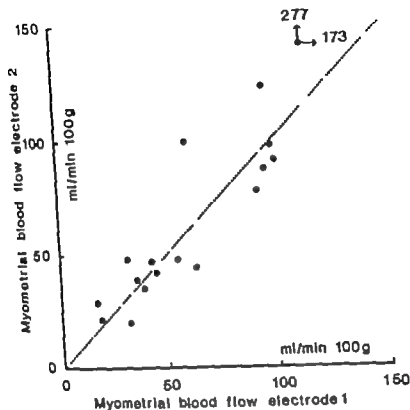


Fig. 4 Comparison between two simultaneous blood flow measurements in the myometrium.

considerably between patients. However if the patients with uterine prolapse are excluded, average myometrial and cervical flow were about the same.

### Discussion

The instrument described for insertion and fixation of electrodes in the myometrium was found to work satisfactorily giving technically good hydrogen desaturation curves from the tissue. The depth of electrode insertion into the myometrium may be regulated, but cannot be exactly localized. Most electrodes used

Table I *Average Myometrial and Cervical Blood Flow*

Diagnosis	Number of Patients	Mean Age	Mean Blood Flow ml/min 100 g	
			Myometrium	Cervix
Uterine and vaginal prolapse	5	63	53	32
Metrorrhagia	4	54.5	57	49
Uterine myomata	3	48.5	98	58
Other	5	36.4	50	57

ments had been made with the electrodes in the same position. Since multiexponential curves are difficult to interpret (Klingenberg and Aukland 1969) the results presented in this paper are based solely on monoexponential curves.

The distribution of mean blood flow obtained from 1-2 electrodes in the myometrium of 22 patients is shown in Fig. 3 upper part giving an average of 64.2 ml/min 100 g. In one patient, blood flow was found to be 225 ml/min 100 g, the others had blood flow varying from 22 to 109 ml/min 100 g. As is evident from Table I, no consistent flow differences were observed between groups of patients with different diagnosis. The high mean flow in the group with uterine myomas is due to high readings (225 ml/min. 100 g) in one patient.

Simultaneous measurements in the myometrium showed good agreement as illustrated in Fig. 4 where values from two electrodes are placed at random on the abscissa and ordinate respectively.

Cervical measurements in 18 patients (Fig. 3 lower part) showed a flow distribution similar to that in the myometrium. Average flow was 47.2 ml/min 100 g, with a range of 17 to 98 ml/min 100 g. In patients with uterine prolapse cervical flow was found to be relatively low. Otherwise no correlation between cervical flow and age or diagnosis could be demonstrated.

Simultaneous measurements in the myometrium and cervix were performed in 17 patients. As is evident from Fig. 5 the relationship between myometrial and cervical blood flow varied

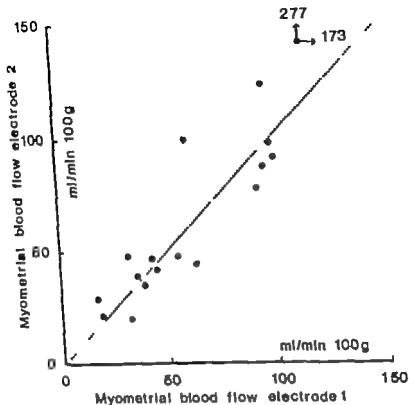


Fig 4 Comparison between two simultaneous blood flow measurements in the myometrium

considerably between patients. However if the patients with uterine prolapse are excluded, average myometrial and cervical flow were about the same

#### Discussion

The instrument described for insertion and fixation of electrodes in the myometrium was found to work satisfactorily giving technically good hydrogen desaturation curves from the tissue. The depth of electrode insertion into the myometrium may be regulated, but cannot be exactly localized. Most electrodes used

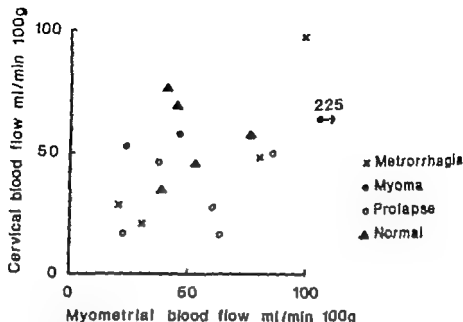


Fig 5. Relationship between blood flow in the myometrium and cervix. Diagnosis indicated by various symbols.

In this investigation were inserted at least 4 mm into the wall, and should therefore have penetrated into the myometrium. However it is possible that due to some irregularity in the shape of the uterine cavity an electrode may have been positioned in the endometrium. The instrument can be introduced through a cervical canal of 4 mm diameter. In parous women, the instrument may be inserted without dilatation of the cervical canal. The measurements were performed with only slight discomfort to the patients and no complications were observed.

Whereas the frequency of monoexponential curves from cervical measurements was about 50 per cent (Klingenberg and Aukland 1969) 70 per cent of the recordings from the myometrium were monoexponential. This may be due to better electrode fixation in the myometrium than in the cervix, and the higher probability of placing the electrodes in the myometrium. The observation may also indicate that blood flow is more homogeneous in the myometrium than in the cervical tissue.

Simultaneous blood flow recordings from two areas in the



myometrium usually showed good agreement. Compared with previous investigations of cervical blood flow (Klingenberg and Aukland 1969) the myometrial scatter was considerably smaller. This suggests that the myometrium has more homogeneous blood flow than the cervix, which is often scarred. The myometrium is probably not completely homogeneous, and some flow variations are therefore to be expected. In the patients in whom relatively large flow differences were observed, one of the electrodes might have been positioned in the endometrium or have been inserted into the peritoneal surface. In contrast, the small difference between simultaneous recordings obtained in most of the patients indicates that both electrodes had been inserted into homogeneous tissue. Since only monoexponential curves were used, it is unlikely that large variations in flow should reflect methodological errors.

As is evident from Table I the blood flow in the myometrium and cervical tissue was of the same magnitude. For unknown reasons the average cervical blood flow obtained in the present patients was somewhat lower than that observed in a previous series (Klingenberg and Aukland, 1969).

Myometrial blood flow showed great variations between patients. The variations in flow obtained in the present study may to some extent be due to differences in diagnosis or age of the patients. However no correlation between flow and diagnosis or age was demonstrated in this material. Variations in flow during the menstrual cycle have not yet been studied. A similar scatter has also been obtained by local  $Xe^{133}$ -clearance technique. In 5 non-pregnant women, Munck *et al.* (1964) found that isthmic flow ranged from 15 to 35 ml/mm 100 g, and flow in the myometrium of pregnant women varied from 4 to 32 ml/min 100 g. Lysgaard and Lefèvre (1965) reported flow values from 2 to 32 ml/min 100 g in women in the last trimester of pregnancy using the same method. With a new instrument for application of  $Xe^{133}$  Lysgaard (1966) reported values varying between 4 and 22 ml/mm 100 g.

Both the present and a previous study (Klingenberg and Aukland 1969) suggest that hydrogen clearance gives higher values than  $Xe^{133}$ -clearance in both the myometrium and the cervix.

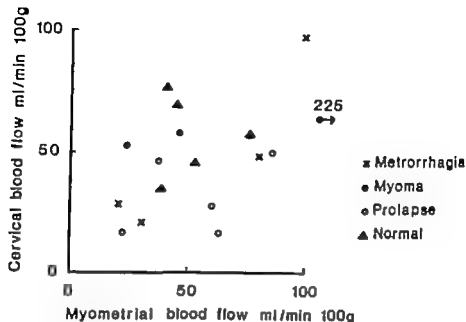


Fig. 5. Relationship between blood flow in the myometrium and cervix. Diagnosis indicated by various symbols.

in this investigation were inserted at least 4 mm into the wall, and should therefore have penetrated into the myometrium. However it is possible that due to some irregularity in the shape of the uterine cavity an electrode may have been positioned in the endometrium. The instrument can be introduced through a cervical canal of 4 mm diameter. In parous women the instrument may be inserted without dilatation of the cervical canal. The measurements were performed with only slight discomfort to the patients and no complications were observed.

Whereas the frequency of monoexponential curves from cervical measurements was about 50 per cent (Klingenberg and Aukland 1969) 70 per cent of the recordings from the myometrium were monoexponential. This may be due to better electrode fixation in the myometrium than in the cervix, and the higher probability of placing the electrodes in the myometrium. The observation may also indicate that blood flow is more homogeneous in the myometrium than in the cervical tissue.

Simultaneous blood flow recordings from two areas in the

## FRICITION BETWEEN THE FOETAL HEAD AND THE CERVIX DURING LABOUR

BY

L. LINDGREN AND D. HOLMLUND

The factors affecting the movements of the foetal head in vertex presentation during labour have been studied by Rydberg (1954). His conclusion was that the shape of the foetal head is a major factor in determining its moving during labour. Internal rotation takes place at different stages during the passage through the pelvis but usually begins when the head approaches the pelvic floor after it has become well flexed. If there is a rotation or not during contraction which causes a gliding between the foetal head and the uterine wall has not been studied as we know from the literature. From Sloner's (1959-1963) investigations we know that during contraction the foetal head is pressed against the lower uterine segment at the same time as the cervix dilates. From these observations the conclusion can be drawn that there is a gliding movement between the foetal head and the uterine wall during contractions. Sloner has also demonstrated that during contraction there is a longitudinal tension in the uterine wall. It is evident that during the contraction there is a friction between the foetal head and the cervical wall, which opposes the moving of the foetal head. It is also evident that the friction must be of great importance for the mechanism of the cervical dilatation during spontaneous delivery and during vacuum extraction when the cervix is not fully dilated.

According to the law of Coulomb the friction ( $F$ ) is the pro-

Although the difference might partly be due to different clinical material, it seems likely that the discrepancy is mainly of methodological origin. As discussed in the previous study acceptance of only monoexponential hydrogen desaturation curves is believed to exclude falsely high values. Furthermore preliminary measurements of blood flow in the uterine artery with an electromagnetic flowmeter have given results compatible with the flow obtained by hydrogen clearance.

### SUMMARY

Blood flow in the human myometrium has been measured by local clearance of hydrogen gas using an instrument that can be introduced through the cervical canal without anaesthesia. The measurements were performed with only slight discomfort to the patients. Monoexponential hydrogen desaturation curves that can be used directly in estimation of myometrial flow were obtained in 22 of 27 women with various gynaecological conditions. Simultaneous measurements from two electrodes introduced into the myometrium showed good agreement but flow values varied widely between patients with an average of 64.2 ml/min 100 g. No significant differences in myometrial blood flow were observed between patients with uterine prolapse, metrorrhagia and uterine myomata.

Supported by a grant from Norsk Forening til Kreftens Bekjempelse.

### REFERENCES

- Munck O, Lysgaard H, Pontonnier G, Lefèvre H and Lassen N A, *Lancet* I 1421 1964  
Lysgaard H and Lefèvre H *Acta obst. et gynec. scandinav* 44 401 1965  
Lysgaard H., *Acta obst. et gynec. scandinav* 45 suppl. 9, 1966  
Klingenberg, I and Aukland K. *Acta obst. et gynec. scandinav* 48 455 1969

Received on April 1, 1968

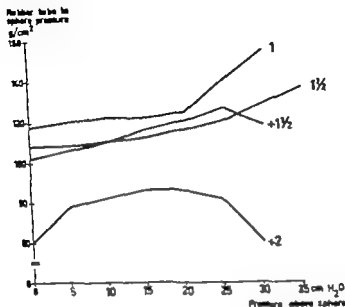


Fig 2 Graph of the sphere to rubber tube pressure at different places of the sphere when increasing pressure was applied above the sphere  
 $+1\frac{1}{2}$  =  $1\frac{1}{2}$  cm above the equator      1 = 1 cm below the equator  
 The curve (+2) is achieved when the tocolograph was applied 2 cm above the equator but with its sensitive part, the cylinder towards the sphere

In order to understand the importance of the pressure conditions and the area of contact between the foetal head and the cervix some model studies have been done. A wooden sphere (8 cm in diameter) corresponding to the foetal head was placed in a rubber tube (6.5 cm in diameter) corresponding to the cervical canal. The pressures between the sphere and the rubber tube at different levels planes on the surface of the sphere were measured by the method for intrauterine tocometry (Ingelman-Sundberg and Lindgren 1953) with different traction forces obtained by means of weights (Fig. 1). At all planes the pressure increased with increased traction but the greatest increase was above the equator of the sphere. The friction is thus increased both as a result of the increased pressure between sphere and rubber tube above the equator and because of an increased area of contact.

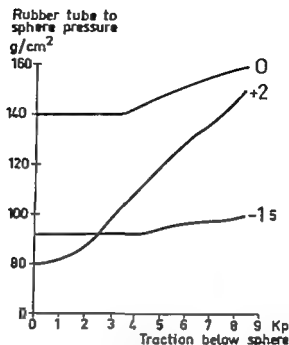


Fig 1 Graph of the sphere to rubber tube pressure at different planes of the sphere when increasing traction was applied below the sphere.  
 0=Equator      +2=2 cm above the equator      -1.5=1.5 cm below the equator

duct of the coefficient of friction ( $f$ ) and the force which presses two objects together ( $N$ )

$$(1) \quad F = f \times N$$

During contraction the friction between foetal head and cervical wall is increased by the driving force which forces the foetal head downwards. This driving force ( $F$ ) is the product of the amniotic fluid pressure ( $P$ ) and in most cases, the cross-section at the largest circumference of the foetal head.

$$(2) \quad F = P \times \pi R^2$$

The factor  $N$  in equation (1) is the product of the head to cervix pressure ( $Q$ ) and the head to cervix area ( $a$ )

$$(3) \quad N = Q \times a$$

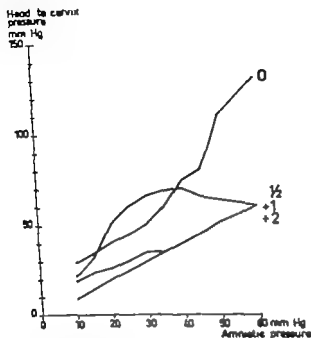


Fig 4 Graph of the head to cervix pressure during contraction. 0—largest circumference of the foetal head +1/2, +1 +2 stands for 1/2, 1 and 2 cm above this level. Two cm above the largest circumference of the foetal head there was no contact between it and the cervical wall.

largest circumference of the foetal head and decreases at the presenting part of the head. During uterine contractions the head to cervix pressure is higher than the corresponding amniotic fluid pressure. This fact explains the engagement of the foetal head in the uterus. During contractions the head to cervix pressure above the equator of the head increases very rapidly (Fig 3) and is sometimes greater than the increase in pressure at the equator (Fig. 4). With further increase in the amniotic fluid pressure the head to cervix pressure at the equator decreases. The amniotic fluid pressure is measured at maximum contraction. This observation shows that the uterine wall above the largest circumference of the foetal head loses contact with the foetal head during contractions.

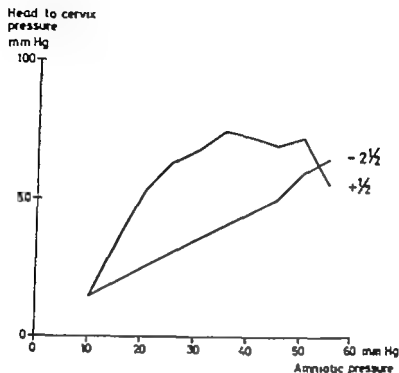


Fig. 3 Graph of the head to cervix pressure during a contraction.  
 $+0.5=0.5$  cm above the largest circumference of the foetal head.  
 $2.5=2.5$  cm below this level.

This observation may have an application in some cases during vacuum extraction when the cervix is not fully dilated.

Water was also poured into the tube from above and the height of the column of water was measured from the top of the sphere by means of a scale outside the rubber tube (Fig. 2). At and below the equator of the sphere an increase in pressure between sphere and rubber tube corresponded to that seen in the traction study. Above the equator a rapid increase of pressure between sphere and rubber tube occurred initially but with an increase in the height of the column of water a decrease in the sphere-rubber tube resulted. This was because the rubber tube above the sphere distended and the area of contact with the sphere decreased. This corresponds with conditions pertaining in normal cervical dilatation.

During labour the head to cervix pressure is greatest at the



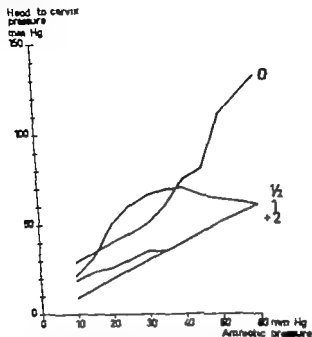


Fig. 4 Graph of the head to cervix pressure during contraction. 0 largest circumference of the foetal head. +1/2, +1 +2 stands for 1/2, 1 and 2 cm above this level. Two cm above the largest circumference of the foetal head there was no contact between it and the cervical wall.

largest circumference of the foetal head and decreases at the presenting part of the head. During uterine contractions the head to cervix pressure is higher than the corresponding amniotic fluid pressure. This fact explains the engagement of the foetal head in the uterus. During contractions the head to cervix pressure above the equator of the head increases very rapidly (Fig. 3) and is sometimes greater than the increase in pressure at the equator (Fig. 4). With further increase in the amniotic fluid pressure the head to cervix pressure at the equator decreases. The amniotic fluid pressure is measured at maximum contraction. This observation shows that the uterine wall above the largest circumference of the foetal head loses contact with the foetal head during contractions.

Table I. *Normal Labour*

Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f
1	I	+	3560	33.5	4.8	25.1	0.19
2	I	+	3090	32	5.0	24.7	0.21
3	I	0	3110	34	10.4	57.4	0.19
4	II	+	3810	35	7.0	33.7	0.21
5	II	0	3740	34	7.6	35.2	0.21

F=Friction N=Resistance of cervical tissue f=coefficient of friction.  
 Estimated in patients with cervical dilatation of 5 cm.

Table II *Spasm of the Lower Uterine Segment*

Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f×N (kp)	D (kp)
1	I	+	3850	37.5	14.4	96.5	19.3	4.9
2	II	0	4200	36	7.8	60.5	12.1	4.3

F=Friction N=Resistance of cervical tissue f=coefficient of friction.  
 D=Difference  
 Estimated in patients with cervical dilatation of 5 cm.

In some cases of normal labour the coefficient of friction has been calculated (Table I). We found that this varied between 0.19 and 0.21 (average 0.2).

In two cases of spasm of the lower uterine segment the driving force was estimated from amniotic fluid pressure and the coefficient of friction. We found in these cases that the driving force was 4.9 and 4.3 kp respectively smaller than the calculated friction (Table II). In such cases there is probably no normal gliding movement between the foetal head and the uterine wall. At maximum contraction the uterine wall is in addition in contact with the foetal head above the largest circumference and this results in increased friction. This friction of the foetal head

explains the delayed or arrested cervical dilatation in such cases. If one tries to dilate the cervix using the vacuum extractor in cases of spasm of the uterine segment the friction increases still more above the largest circumference as in the model study by traction and cervical dilatation is impossible. Sometimes the same effect may occur in normal labour if the normal increase in width of the uterus upwards is not present. A similar situation may arise in cases of oligohydramnios especially when traction is applied between contractions.

### SUMMARY

In vertex presentation the friction between the foetal head and the uterine wall has been studied by means of intrauterine tocometry. The results have been compared with those in model studies using a wooden sphere in a rubber tube. In normal contractions the uterine wall above the largest circumference of the foetal head (the equator of the foetal head) loses contact with the foetal head as the rubber tube above the equator of the sphere when the rubber tube is filled by a column of water. The friction decreases above the equator in both cases. In lower uterine spasm and by traction of the sphere by weights the friction increases above the equator during contraction. This observation may explain the poor effect by using vacuum extractor in lower uterine spasm and in oligohydramnios.

### REFERENCES

- Holmhead D. *Scandinav J Urology & Nephrology Suppl.* 1 27 1968  
 Holmhead, D and Lindgren L. Friction between foetal head and uterine wall during normal labour and during lower uterine spasm. *Am J Obst & Gynec* 103 939 1969  
 Ingelman-Sundberg, A and Lindgren L. *J Obst & Gynaec Brit Emp* 62 629 1955  
 Lindgren L. *Acta obst et gynec scandinav Suppl* 2 34 1955  
*Nord Med* 85 541 1961  
 Rydberg, E. *The mechanism of labour* Charles C Thomas publisher Springfield, USA, 1954  
 Sauer H. *Geburts- und Frauenheilk* 19 140 1959  
*Am J Obst & Gynec* 86 303 1963  
 Received on Oct 4, 1968

Table I. *Normal Labour*

Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f
1	I	+	3560	33.5	4.8	25.1	0.19
2	I	+	3090	32	5.0	24.7	0.21
3	I	0	3110	34	10.4	57.4	0.19
4	II	+	3810	35	7.0	33.7	0.21
5	II	0	3740	34	7.6	35.2	0.21

F=Friction N=Resistance of cervical tissue f=coefficient of friction.  
 Estimated in patients with cervical dilatation of 5 cm.

Table II. *Spasm of the Lower Uterine Segment*

Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f \ N (kp)	D (kp)
1	I	+	3850	37.5	14.4	96.5	19.3	4.9
2	II	0	4200	36	7.8	60.5	12.1	4.3

F=Friction N=Resistance of cervical tissue f=coefficient of friction.  
 D=Difference  
 Estimated in patients with cervical dilatation of 5 cm.

In some cases of normal labour the coefficient of friction has been calculated (Table I). We found that this varied between 0.19 and 0.21 (average 0.2).

In two cases of spasm of the lower uterine segment the driving force was estimated from amniotic fluid pressure and the coefficient of friction. We found in these cases that the driving force was 4.9 and 4.3 kp respectively smaller than the calculated friction (Table II). In such cases there is probably no normal gliding movement between the foetal head and the uterine wall. At maximum contraction the uterine wall is in addition in contact with the foetal head above the largest circumference and this results in increased friction. This friction of the foetal head

explains the delayed or arrested cervical dilatation in such cases. If one tries to dilate the cervix using the vacuum extractor in cases of spasm of the uterine segment the friction increases still more above the largest circumference as in the model study by traction and cervical dilatation is impossible. Sometimes the same effect may occur in normal labour if the normal increase in width of the uterus upwards is not present. A similar situation may arise in cases of oligohydramnios especially when traction is applied between contractions.

### SUMMARY

In vertex presentation the friction between the foetal head and the uterine wall has been studied by means of intrauterine tocometry. The results have been compared with those in model studies using a wooden sphere in a rubber tube. In normal contractions the uterine wall above the largest circumference of the foetal head (the equator of the foetal head) loses contact with the foetal head as the rubber tube above the equator of the sphere when the rubber tube is filled by a column of water. The friction decreases above the equator in both cases. In lower uterine spasm and by traction of the sphere by weights the friction increases above the equator during contraction. This observation may explain the poor effect by using vacuum extractor in lower uterine spasm and in oligohydramnios.

### REFERENCES

- Holmlund P. *Scandinav J Urology & Nephrology* Suppl 1 27 1968  
 Holmlund D and Lindgren L. Friction between foetal head and uterine wall during normal labour and during lower uterine spasm. *Am J Obst & Gynec* 103 939 1969  
 Leprman-Sundberg, A and Lindgren L. *J Obst & Gynaec, Brit. Emp* 62 629 1955  
 Lindgren L. *Acta obst. et gynec. scandinav* Suppl 2, 34 1955  
 Nord Med 65 541 1961  
 R. Järg E. *The mechanism of labour* Charles C Thomas publisher Springfield, USA 1954  
 Sæver H. *Geburts- und Frauenheilk.* 19 140 1959  
*Am J Obst & Gynec* 86 303 1963  
 Received on Oct 4 1968

## BROW PRESENTATIONS

BY

ARNI INGOLFSSON

The present paper concerns cases of brow presentation and related deflexed attitudes (between brow and face and brow and median vertex) that occurred in the Department of Obstetrics and Gynaecology in Lund during the years 1932 to 1967. During this period 88,988 deliveries took place at the department (Table I). The frequency of brow presentation was 1.15% i.e. about one in 860 and during 1900–1931 the incidence was one in 800 deliveries (Sjövall 1933). The frequency has obviously been quite constant during the past 67 years.

The frequency of brow presentation in our department is somewhat higher than in most other reports. The department receives all parturients in the area served by the hospital except those who are delivered in the small maternity units in nearby hospitals. Since 1958 there have been practically no home deliveries at all in the country. The material presented here comprises 91 cases of brow presentation, 6 of brow face presentation and 6 of brow median vertex presentation.

### *Aetiological Factors*

The factors leading to brow presentation are considered to be the same as those causing deflexion attitudes in general. The question is why the head adopts precisely this intermediate attitude between vertex and face presentation.

Too early drainage of the amniotic fluid i.e. rupture of the membranes before labour has begun has previously been con-

considered an important cause of deflexion attitudes. It is just as plausible, however, that too early drainage of amniotic fluid is caused by the deflexion, the foetal head being less well adapted in the pelvis. In 27 cases—26 per cent—early rupture of the membranes occurred.

Disproportion between the pelvis and the foetal head can cause deflexion. Disproportion was considered to be present in 16 cases (15.3 per cent) in 3 cases owing to a too big child.

Ample space between the foetal head and the pelvic walls may also result in brow presentation. Six infants (5.8 per cent) were premature, with a birth weight of less than 2500 g.

It is not unusual for twins to be born with the head more or less deflexed. Between 1932 and 1967 1176 twins were delivered in our department. Of these, 7 (0.6 per cent) were born in brow presentation.

Multiparity has been thought to be one of the most important causes of deflexion attitudes, due to slackness in the abdominal muscles, possibly in association with uterine inertia. In this series (Table 1) 51 of the brow presentations occurred in *primiparae* (1.35 %) and 52 (1.02 %) in *multiparae*. Brow presentation were thus somewhat more common in *primiparae*.

Borell and Fernström (1960) believe that the reason the head persists in brow presentation probably is that the mouth of the foetus is open and the foetal chin is pressed against the chest. Borell and Fernström also describe a method to correct the brow presentation to face presentation by closing the mouth of the foetus. In our series in one instance X-ray showed presentation with the mouth of the foetus open.

Table 1 Frequency of Brow Presentation in *Primiparae* and *Multiparae* (1932-1967)

Grade	Deliveries	Brow Presentation	Per 1000 Deliveries
Primiparae	37,902	51	1.35
Multiparae	51,066	52	1.02
Total	88,968	103	1.15

## BROW PRESENTATIONS

BY

ARNI INGOLFSSON

The present paper concerns cases of brow presentation and related deflexed attitudes (between brow and face, and brow and median vertex) that occurred in the Department of Obstetrics and Gynaecology in Lund during the years 1932 to 1967. During this period, 88 988 deliveries took place at the department (Table I). The frequency of brow presentation was 1.15 %, i.e. about one in 860, and during 1900–1931 the incidence was one in 800 deliveries (Sjövall 1933). The frequency has obviously been quite constant during the past 67 years.

The frequency of brow presentation in our department is somewhat higher than in most other reports. The department receives all parturients in the area served by the hospital, except those who are delivered in the small maternity units in nearby hospitals. Since 1958 there have been practically no home deliveries at all in the country. The material presented here comprises 91 cases of brow presentation: 6 of brow-face presentation and 6 of brow-median vertex presentation.

### *Aetiological Factors*

The factors leading to brow presentation are considered to be the same as those causing deflexion attitudes in general. The question is why the head adopts precisely this intermediate attitude between vertex and face presentation.

Too early drainage of the amniotic fluid, i.e. rupture of the membranes before labour has begun, has previously been con-



Table IV Duration of Labour in Hours (Average)

Gravida	Vaginal	Cæsarean Section
Primipara	27 2/3	18 1/3
Multipara	14	7 1/2

Table V Prolonged Labour in Brow Presentation

Gravida	Prolonged Labour	Per 100 Deliveries
Primipara	22	43
Multipara	21	40
Total	43	41.5

More than 18 hours in primiparae and 12 hours in multiparae.

### Clinical Course

According to various reports the delivery in brow presentation is spontaneous in between 12 and 44 per cent. In the present series 46 cases (45 per cent) were delivered spontaneously.

As can be seen in Tables IV and V prolonged labour is very common. In the tables, an upper limit is set at 18 hours as normal for primigravidae and 12 hours for multigravidae (i.e. a woman with earlier labour). Prolonged labour occurred in 43 per cent of primigravidae and in 40 per cent of multigravidae. Of all deliveries 21 (20 per cent) took 30 hours or more. In eight cases, delivery lasted for five hours or less. All eight were multigravidae except one (premature delivery 1350 g).

From this it can be concluded that brow presentation labours of short duration are very unusual among primigravidae and that prolonged labours are very common in both primigravidae and multigravidae. The reason for the prolonged labour is most often dystocia. To a lesser extent uterine inertia, also plays a part. 27 cases showed signs of inertia, 16 being recorded as primary and 11 as secondary.

Cranial anomalies in the foetus can produce deflexion of the foetal head. Our series contains two instances of anencephalic monsters.

In 22 cases, the umbilical cord was twisted once or several times round the neck of the child. There is also one instance of polyhydramnios, one of prolapse of the cord, and one of placenta praevia. In two instances, there were fibroids in the lower part of the uterus.

In four cases external version of breech presentation had been made during pregnancy and in two from transverse lie (5.8 per cent). Consequently it seems that external version may cause deflexion of the foetal head.

### *Diagnosis*

Table II shows the methods used for diagnosis and Table III at the stage of delivery when the diagnosis was made. The diagnosis was made late in a large percentage of the cases in about 47 per cent, not until delivery. This agrees with other reports. In the spontaneous deliveries the diagnosis of brow presentation was not made until delivery in 78 per cent.

Table II. *Brow Presentation and Methods of Making the Diagnosis*

Method	Number of Patients
Rectal examination	7
Vaginal examination	2
X-ray examination	26
At delivery	48
Total	103

Table III. *The Stage of Labour when the Diagnosis of Brow Presentation Was Made*

Stage I	32
Stage II	23
At delivery	48
Total	103

Table IV Duration of Labour in Hours (Average)

Gravida	Vaginal	Caesarean Section
Primigravida	27 2/3	18 1/3
Multigravida	14	7 1/2

Table V Prolonged Labour in Brow Presentation

Gravida	Prolonged Labour	Per 100 Deliveries
Primigravida	22	43
Multigravida	21	40
Total	43	41.5

More than 18 hours in primigravidae and 12 hours in multigravidae.

### Clinical Course

According to various reports the delivery in brow presentation is spontaneous in between 12 and 44 per cent. In the present series 46 cases (45 per cent) were delivered spontaneously.

As can be seen in Tables IV and V prolonged labour is very common. In the tables an upper limit is set at 18 hours as normal for primigravidae and 12 hours for multigravidae (i.e. a woman with earlier labour). Prolonged labour occurred in 43 per cent of primigravidae, and in 40 per cent of multigravidae. Of all deliveries 21 (20 per cent) took 30 hours or more. In eight cases delivery lasted for five hours or less. All eight were multigravidae except one (premature delivery 1350 g).

From this it can be concluded that brow presentation labours of short duration are very unusual among primigravidae and that prolonged labours are very common in both primigravidae and multigravidae. The reason for the prolonged labour is most often dystocia. To a lesser extent uterine inertia, also plays a part. 27 cases showed signs of inertia, 16 being recorded as primary and 11 as secondary.

Cranial anomalies in the foetus can produce deflexion of the foetal head. Our series contains two instances of anencephalic monsters.

In 22 cases the umbilical cord was twisted once or several times round the neck of the child. There is also one instance of polyhydramnios one of prolapse of the cord, and one of placenta praevia. In two instances there were fibroids in the lower part of the uterus

In four cases external version of breech presentation had been made during pregnancy and in two from transverse lie (5.8 per cent) Consequently it seems that external version may cause deflexion of the foetal head.

### *Diagnosis*

Table II shows the methods used for diagnosis and Table III at the stage of delivery when the diagnosis was made. The diagnosis was made late in a large percentage of the cases in about 47 per cent, not until delivery. This agrees with other reports. In the spontaneous deliveries the diagnosis of brow presentation was not made until delivery in 78 per cent

*Table II. Brow Presentation and Methods of Making the Diagnosis*

Method	Number of Patients
Rectal examination	7
Vaginal examination	22
X-ray examination	26
At delivery	48
Total	103

*Table III. The Stage of Labour when the Diagnosis of Brow Presentation Was Made*

Stage I	32
Stage II	23
At delivery	48
Total	103

**B. Forceps or vacuum extraction** 26 patients were delivered by forceps or vacuum extraction.

Seventeen of the children were in fronto-anterior position when the intervention was begun. In two the foetal head was rotated to occipito-anterior position. In one case correction to face presentation was attempted but failed.

In two cases the heads were in fronto-transverse position and both were rotated to fronto-anterior position.

Four children were in fronto-posterior position. Two were rotated to fronto-anterior position, the other two were corrected to occipito-posterior position before extraction.

In three instances the position was not recorded with sufficient exactness in the case notes.

**C. Craniotomy and cranioclasty** Craniotomy and cranioclasty were performed on dead foetuses in three cases.

The first, from 1939 concerned a primigravida with total of 23 hours labour pains and fever. The last 40 hours prior to delivery no foetal heart sounds were audible. The foetus was extracted in fronto-anterior position.

The second, from 1957 concerned a primigravida with labour pains for 20 hours. The foetal heart was no longer audible about 4 hours before delivery. The position was fronto-anterior.

The third, from 1967 concerned a gravida IV with labour pains for about 8 hours. On admission the labour pains were moderate. Immediately after the foetal heart sounds were established as normal but ten minutes later no longer were audible. At vaginal examination fronto-posterior position was diagnosed. At full dilatation attempts were made to correct the position of the head, but when this failed, craniotomy and cranioclasty were performed.

**D. Podalic version and extraction** This intervention was performed in two instances.

The first, in 1947 concerned a gravida III with labour pains for 18.5 hours. The exact position of the foetal head is not stated in the case record. Before version efforts to flex the head as well as attempts to deflex the head to face presentation and apply forceps and make extraction had failed. In spite of this living child weighing 4150 g was delivered by version and extraction.

The second case in 1953 was a gravida III with labour pains for 9.25 hours. The foetal head was in fronto-transverse position. Attempts to rotate the head failed. Attempts to flex the head and to apply forceps in order

Table VI. Position of the Foetal Head at Spontaneous and Operative Delivery

Partus	Fronto-anterior	Fronto-transverse	Fronto-posterior	Undetermined	Total	
					Number	Per Cent
Spontaneous	42	2	2		46	45
Forceps or VE	17	?	4	3	26	25
Caesarean section	7	4	4	10	25	24
Craniotomy and Cranioclasty	2		1		3	3
Version and extraction after failed forceps		1		1	2	?
Hysterectomy on ac- count of uterine rupture				1	1	1
Total	68	9	11	15	103	100

Of those delivered in fronto-posterior position, one, weighing 3'00 g, was the child of a secundigravida. X-ray pelvimetry a few days after delivery showed a true conjugate of 12.5 cm.

The mother of the other child was a primigravida. This child also weighed 3'00 g. It was born in a presentation intermediate between fronto-posterior and median vertex presentation. Both the children were living.

### Mode of Delivery

*A. Spontaneous deliveries* Delivery occurred spontaneously in 46 cases. 42 of the children were born with the head in the fronto-anterior position, two in the fronto-transverse and two in the fronto-posterior position.

Of those delivered in fronto-posterior position, one, weighing 3'00 g, was the child of a secundigravida. X-ray pelvimetry a few days after delivery showed a true conjugate of 12.5 cm.

The mother of the other child was a primigravida. This child also weighed 3'00 g. It was born in a presentation intermediate between fronto-posterior and median vertex presentation. Both the children were living.

Cases of spontaneous delivery in fronto-posterior presentation have been reported previously (Johansson 1945, Ingerslev 1951) and it should be stressed that this can occur also when the child is rather big. Most of those children were also living.

Table VII. *Indications for Caesarean Section*

Indications	Number of Patients	Percentage
Dysproportion (pelvis/head)	14	56
Prolonged labour+arrest	5	20
Other causes (Placenta praevia, elderly primigravidae etc.)	6	24
Total	25	100

Table VIII. *Summary of Spontaneous Deliveries and Mortality*

Spontaneous delivery	46 patients
Discharged living	38 infants
Dead at delivery	8 infants
Of these,	
Anencephalus	2 infants
Erythroblastosis	2 infants
Mongolism	1 infant
Corrected mortality	3 infants (7 per cent)

Table IX. *Summary of Forceps and VE Operations and Perinatal Mortality*

Forceps and VE operations	26 patients
Discharged living	21 infants
Dead at delivery	5 infants
Of these,	
Premature (1350 g) (30th week)	1 infant
Corrected mortality	4 infants (16 per cent)

Table X. *Brow Presentations Frequency of Caesarean Sections and Perinatal Mortality at Different Periods*

Period	Number of Deliveries	Frequency of Caesarean Section (per cent)	Corrected Perinatal Mortality (per cent)
1900-1931 (Sjovall 1933)	46	2	21
1932-1950	53	8	18
1951-1967	50	45	6
(1958-1967)	30	54	3

to extract the child failed. After podalic version a dead child weighing 4500 g was extracted.

In the series there is also a case of uterine rupture from the year 1944

Gravida VI, 38 years of age. Five previous normal deliveries, all infants weighing between 4000 and 5000 g. Labour pains for 101 hours. X-ray on the second day of labour showed deflexion attitude between median vertex and brow presentation. Approximately 24 hours before delivery no foetal sounds were audible. The patient suddenly felt pain over the symphysis and became pale and ill. Laparotomy was immediately carried out. The rupture of the uterus included also the upper part of the vagina. A subtotal hysterectomy was carried out as well as suture of the vaginal rupture. The patient died at the end of the operation. The foetus was dead, it weighed 5200 g.

*E Caesarean section.* Von Hireniger-Guggenberger suggested (1930) that Caesarean section should be resorted to more often in brow presentations.

The frequency of Caesarean section in cases of brow presentation has, according to various authors risen successively. Whereas Sjövall (1933) reported a frequency of only two per cent for 46 cases during the years 1900-1931 and Ingerslev four per cent for 200 cases (1951) Magid and Gillespie (1957) have a figure of 37.5 per cent and Jacobson and Johnsson (1962) 18 per cent.

Dividing our material into two periods (Table X) gives a frequency of Caesarean section of 8 per cent before 1950 and 45 per cent after. During the past ten years, Caesarean section was undertaken in no less than 15 cases (over 50 per cent). The tendency at our department is to perform Caesarean section more and more often for brow presentation. Table VII shows the indications for Caesarean section. Of the Caesarean section cases one was a gravida II with brow presentation on both occasions the patient was operated upon by Caesarean section in both instances.

### *Perinatal Mortality*

In the literature figures for infant mortality vary between six per cent and 25 per cent. In our series the corrected mortality is 12 cases. This is a high figure compared with the general per-



Table VII. *Indications for Caesarean Section*

Indication	Number of Patients	Percentage
Disproportion (pelvis/head)	14	58
Prolonged labour + arrest	5	20
Other causes (Placenta praevia, elderly primigravidae etc.)	6	24
Total	25	100

Table VIII. *Summary of Spontaneous Deliveries and Mortality*

Spontaneous delivery	46 patients
Discharged living	38 infants
Dead at delivery	8 infants
Of these	
Anencephalus	2 infants
Erythroblastosis	2 infants
Mongolian	1 infant
Corrected mortality	3 infants (7 per cent)

Table IX. *Summary of Forceps and VE Operations and Perinatal Mortality*

Forceps and VE operations	28 patients
Discharged living	21 infants
Dead at delivery	5 infants
Of these	
Premature (1350 g.) (30th week)	1 infant
Corrected mortality	4 infants (16 per cent)

Table X. *Brow Presentations Frequency of Caesarean Sections and Perinatal Mortality at Different Periods*

Period	Number of Deliveries	Frequency of Caesarean Section (per cent)	Corrected Perinatal Mortality (per cent)
1900-1931 (Sjövall 1933)	46	2	21
1932-1950	53	8	18
1951-1967	50	45	6
(1958-1967)	30	54	3

to extract the child failed. After podalic version a dead child weighing 4300 g was extracted.

In the series there is also a case of uterine rupture from the year 1944

Gravida VI 38 years of age. Five previous normal deliveries all infants weighing between 4000 and 5000 g. Labour pains for 101 hours X-ray on the second day of labour showed deflexion attitude between median vertex and brow presentation. Approximately 24 hours before delivery no foetal sounds were audible. The patient suddenly felt pain over the symphysis and became pale and ill. Laparotomy was immediately carried out. The rupture of the uterus included also the upper part of the vagina. A subtotal hysterectomy was carried out as well as suture of the vaginal rupture. The patient died at the end of the operation. The foetus was dead, it weighed 5200 g

*E. Caesarean section* Von Khreninger-Guggenberger suggested (1930) that Caesarean section should be resorted to more often in brow presentations.

The frequency of Caesarean section in cases of brow presentation has according to various authors, risen successively. Whereas *Sjövall* (1933) reported a frequency of only two per cent for 46 cases during the years 1900–1931 and *Ingerslev* four per cent for 200 cases (1951) *Magid* and *Gillespie* (1957) have a figure of 37.5 per cent and *Jacobson* and *Johnsson* (1962) 18 per cent.

Dividing our material into two periods (Table X) gives a frequency of Caesarean section of 8 per cent before 1950 and 45 per cent after. During the past ten years Caesarean section was undertaken in no less than 15 cases (over 50 per cent). The tendency at our department is to perform Caesarean section more and more often for brow presentation. Table VII shows the indications for Caesarean section. Of the Caesarean section cases one was a gravida II with brow presentation on both occasions the patient was operated upon by Caesarean section in both instances.

### *Perinatal Mortality*

In the literature figures for infant mortality vary between six per cent and 25 per cent. In our series the corrected mortality is 12 cases. This is a high figure compared with the general peri-

in 45 per cent. During the past ten years Caesarean section was performed in over 50 per cent and the corrected perinatal mortality during this period was three per cent (only the cranio-tomy case from 1967). It is thus obvious that the prognosis for the child in brow presentation has successively improved with the increase in the Caesarean section rate (Table X).

However the question remains whether as soon as a brow presentation is diagnosed Caesarean section should always be resorted to or expectancy might be permitted.

It should be kept in mind that brow presentation can change spontaneously to face- or even vertex presentation during the course of labour.

Forty-six of the deliveries in brow presentation were spontaneous and most of those who required termination by forceps or vacuum extraction were successful. On the other hand those spontaneous deliveries, forceps and vacuum extractions had a rather high perinatal mortality seven per cent and 16 per cent respectively. The conclusion seems therefore to be that expectancy might be permitted only under very favourable conditions such as a roomy pelvis, a not too big foetus, excellent uterine contractions and good progress of labour. As soon as labour does not progress or even moderate complications occur Caesarean section should be undertaken. High and mid forceps operations and especially podalic version and extraction should be avoided, whereas outlet forceps or vacuum extraction in brow presentation can be made. Simple methods of manual correction such as that described by *Borell* and *Fernström* should also be considered in suitable cases.

### SUMMARY

The 103 cases of brow presentation which occurred at the Department of Obstetrics and Gynaecology in Lund during the years 1932-1967 are reviewed, especially regarding the perinatal mortality.

Spontaneous deliveries in brow presentation occurred in 45 per cent, forceps or vacuum extraction in 25 per cent and Caesarean section in 24 per cent.

Table XI. *Perinatal Mortality Before and after 1950*

Up to and Including 1950	1950 Onwards
Total 53 patients	Total 50 patients
Discharged living 41 infants (77 per cent)	Discharged living 44 infants (88 per cent)
Perinatal deaths 12 (23 per cent)	Perinatal deaths 6 (12 per cent)
Of the 12,	Of the 6
1 mongoloid	1 erythroblastosis (intrauterine death)
1 anencephalus	1 anencephalus
1 erythroblastosis	1 premature (1350 g) (30th week)
Corrected mortality 9 infants (18 per cent)	Corrected mortality 3 infants (6 per cent)

natal mortality in the department during the years 1945-1967. At the beginning of this period it was 2.7 per cent and has slowly fallen to 1.8 per cent in 1967. This demonstrates how unfavourable brow presentation is for the foetus. The spontaneous deliveries in brow presentation (Table VIII) show a corrected mortality of 7 per cent. If however the two craniotomy cases and the uterine rupture case—all of which were caused by unduly prolonged expectancy—are added to the spontaneous deliveries a total of six dead children (approximately 13 per cent) is obtained.

In the forceps and vacuum extraction operations (Table IX) the corrected mortality is 16 per cent.

Podalic version and extraction was carried out in two cases after failed attempts at correction and forceps extraction. One of the children died.

In the Caesarean section cases there was no foetal mortality

### Discussion

The entire series is divided as shown in Table XI into two periods. It can be seen that the perinatal mortality before 1950 was 18 per cent and after six per cent. Before 1950 Caesarean section was undertaken in eight per cent of the cases, after 1950

## THE URINARY EXCRETION OF OESTRIOL IN PREGNANCIES WITH RH IMMUNISATION

BY

F. LUNDVALL AND G. STAKEMANN

Estimation of the urinary excretion of oestriol has received increasing interest from obstetricians. It seems to reflect the condition of the foetus and in this way it can be used as a guide in the treatment of various pathological conditions. Low or declining values appear to indicate that danger to the foetus is present and extremely low values indicate foetal death.

In pregnancies complicated by Rh-immunisation reports are somewhat conflicting. In such cases Taylor *et al.* (1963) found a higher output of oestriol than in normal pregnancies. In contrast Kloppe and Stephenson (1966) in 49 cases, found values within the normal range even in severely sensitized cases. Barerjes (1962) found high values in a moderately sensitized patient. In another where the foetus died in utero he found low values. Schindler *et al.* (1967) found that the concentration of oestriol in the amniotic fluid reflected the condition of the foetus better than either the concentration in plasma or the urinary excretion.

The present investigation is a retrospective analysis of the correlation between the foetal status and the maternal urinary excretion of oestriol in a rather large series of patients.

### Method

All hormone estimations were carried out at the hormone department of the Serum Institute using the method developed by F. Andersen (1963)

Podalic version and extraction after failed forceps was made in two cases.

Craniotomy was required in three cases.

One mother died. This occurred during performance of a sub-total hysterectomy for rupture of the uterus.

The corrected total perinatal mortality was 12.4 per cent. It has successively fallen from 18 per cent during 1932-1950 to 5 per cent after 1950 and during the last ten years to 3 per cent along with an increase in the Caesarean section rate from 8 per cent to 45 per cent and over 50 per cent respectively.

In very favourable cases of brow presentation spontaneous delivery or easy outlet extraction can be considered, in others Caesarean section should be undertaken.

#### REFERENCES

- Borell U and Fernström I *Acta obst. et gynec. scandinav* 39 626 1960  
Ingerslev M *Acta obst. et gynec. scandinav* 30 278 1951  
Jacobson L. J and Johansson C E, *Am. J Obst. & Gynec.* 84 1881 1962  
Johansson H *Monatsschr Geburtsh.* 120 121 1945  
v Khreninger-Guggenberger J *Arch. f Gynäk.* 142 197 1930  
Magid, B and Gillespie C. F *Obst. & Gynec.* 9 450 1957  
Meltzer R. M., Sachleben M. R. and Friedman E. A. *Am. J Obst. & Gynec.* 100 255 1968  
Posner L. B. Rubin E. J and Posner A. C., *Obst. & Gynec.* 21 745 1963  
Sjövall A. *Hygiea* 95 341 1933  
- *Gynec. et obst.* 30 326 1933  
Received on Aug. 7 1968

## THE URINARY EXCRETION OF OESTRIOL IN PREGNANCIES WITH RH IMMUNISATION

BY

F LUNDVALL AND G STAKEMANN

Estimation of the urinary excretion of oestriol has received increasing interest from obstetricians. It seems to reflect the condition of the foetus and in this way it can be used as a guide in the treatment of various pathological conditions. Low or declining values appear to indicate that danger to the foetus is present and extremely low values indicate foetal death.

In pregnancies complicated by Rh-immunisation reports are somewhat conflicting. In such cases Taylor *et al* (1963) found a higher output of oestriol than in normal pregnancies. In contrast Kloppe and Stephenson (1966) in 49 cases found values within the normal range even in severely sensitized cases. Bowerjee (1962) found high values in a moderately sensitized patient. In another where the foetus died in utero he found low values. Schindler *et al* (1967) found that the concentration of oestriol in the amniotic fluid reflected the condition of the foetus better than either the concentration in plasma or the urinary excretion.

The present investigation is a retrospective analysis of the correlation between the foetal status and the maternal urinary excretion of oestriol in a rather large series of patients.

### Method

All hormone estimations were carried out at the hormone department of the Serum Institute using the method developed by Frandsen (1963)

### Material

At the Rigshospitalet obstetrical department A 156 consecutive patients with Rh immunisation were investigated. Only a few cases had to be excluded because the patients were uncertain of the date of their last menstrual period. Values obtained after obvious foetal death have been excluded. The material has been divided into two groups one where Coombs test on cord blood indicated Rh affected babies and another where no such affection could be demonstrated. The first group consisted of 122 patients the second of 34.

Twenty-four hours urine specimens were collected at weekly intervals during the last month before calculated term, and in many cases samples were also collected eight and twelve weeks before term. Treatment of the patients was in no way influenced by the results of the hormone analyses.

The obstetrical management was based on the estimated degree of immunisation as judged by the results of repeated indirect Coombs tests during pregnancy and by the past obstetrical history. Intrauterine transfusions were not used during the period under review but in the more severely affected cases labour was induced or Caesarean section carried out when the foetal weight was estimated to be 2800 g i.e. two weeks before term. Fifty-nine women had labour induced and five had Caesarean section.

The degree of Rh affection of the babies as judged from the concentration of haemoglobin in the cord blood is shown in Table I.

Table I. Haemoglobin-concentration in Cord Blood

g/100 ml	Number of Babies	
	Rh affected	Rh unaffected
>17.8	13 (11)	12 (35)
<17.8-14.8	37 (30)	18 (53)
<14.8-11.8	46 (38)	4 (12)
<11.8	26 (21)	0 (0)

Figures in brackets show percentages



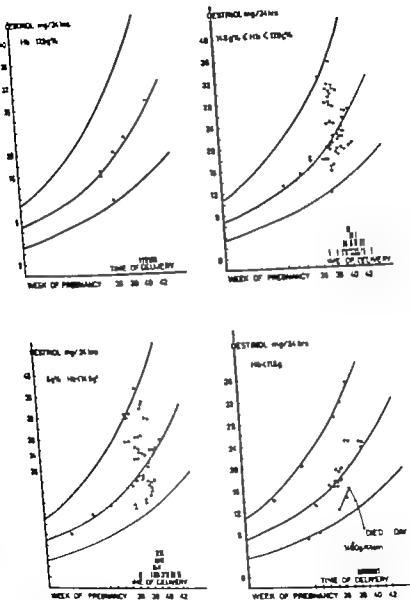


Fig 1 Excretion of oestrol in 122 Rh-immunized patients delivered of affected babies.

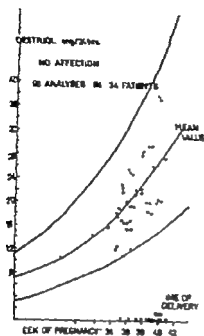


Fig. 2. Excretion of oestradiol in 34 Rb-immunized patients delivered of unaffected babies

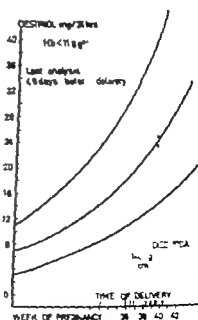
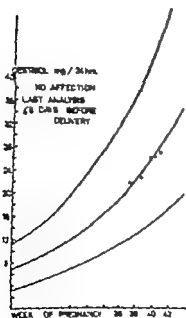


Fig. 3. The oestradiol excretion estimated during the last week before delivery in patients delivered of unaffected and of severely affected babies.

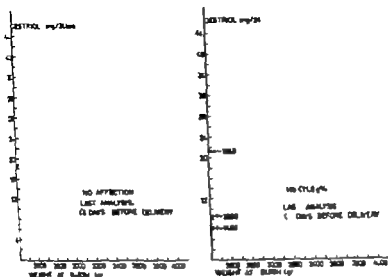


Fig 4 The values of Fig 3 in relation to birth weight

### Results

Figure 1 shows all the values in the series according to the concentration of haemoglobin in cord blood. The lines indicate the range and average excretion of oestriol in normal pregnancies (from Frandsen and Strakemann 1963). The oestriol output in Rh-immunized patients shows a distribution similar to that of normal pregnancies even in cases where the baby was severely affected. For comparison is shown the excretion of patients where the babies were unaffected (Fig. 2).

It might be that a difference in the hormone excretion between mild and severe cases was present only shortly before term. Figure 3 shows a comparison of the hormone excretion estimated less than one week before delivery in patients with unaffected babies and those with severely affected babies. No significant difference can be found.

It has been shown that a correlation exists between the weight of the baby and the amount of oestriol excreted (Frandsen and

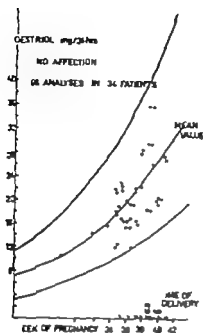


Fig. 2. Excretion of oestriol in 34 Rh-immunized patients delivered of unaffected babies.

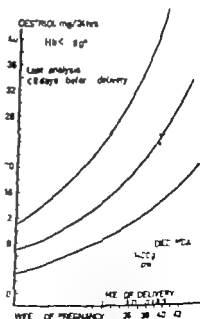
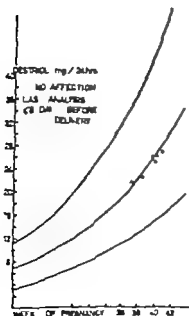


Fig. 3. The oestriol excretion estimated during the last week before delivery in patients delivered of unaffected and of severely affected babies.

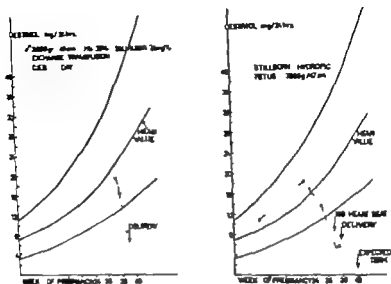


Fig. 6 Oestrol excretion in two patients in which the life of the foetus was severely jeopardized.

Stakemann 1960 Coyle and Brown 1963) The values from Figure 3 are therefore shown in relation to the birth weight of the baby in Figure 4

Again no difference can be demonstrated between patients with unaffected and patients with severely affected babies.

Another parameter for evaluation of the degree of erythroblastosis in the baby is the number of exchange transfusions necessary

In Fig. 5 the series has been analysed according to the number of exchange transfusions given

The results show no significant difference in the oestrol excretion just before delivery in cases needing none, one, two or three or more transfusions.

Although Rh-immunisation *per se* apparently does not influence the maternal urinary excretion of oestrol a low output can be demonstrated in cases where the life of the foetus is severely jeopardized. Fig. 6 shows two such cases.

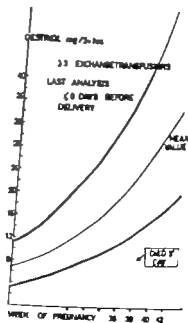
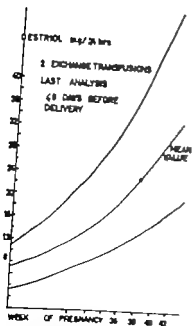
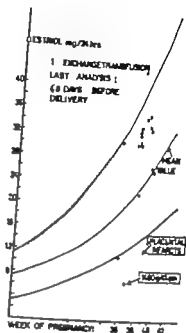
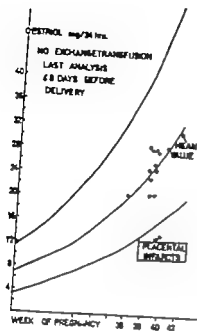


Fig. 5 Comparison of oestriol excretion in relation to the number of exchange transfusions needed.

## COAGULATION AND FIBRINOLYSIS IN PREMATURELY DELIVERED MOTHERS AND THEIR PREMATURE INFANTS

By

NIELS CHR. NIELSEN

In a number of recent communications it has been confirmed that the content of several coagulation factors in the blood increases as pregnancy advances. Fibrinolysis during pregnancy has also been thoroughly studied.

Coagulation and fibrinolysis in premature babies have been studied in a number of publications. However it is difficult to compare the studies, because the blood samples were taken at different times after delivery and because different techniques were used. Moreover the term prematurity is usually based only upon the birth weight (under 2500 g) without paying regard to gestational age.

The object of the present work was to study coagulation and fibrinolysis in a well-defined group of infants born more than four weeks before term in whom the blood samples were removed under standardized conditions. The results will be compared with those obtained by analyses on maternal blood.

This material allows subsequent comparison with similar findings in premature babies from pregnancies complicated by e.g. to rhesus iso-immunization, pre-eclampsia, and abruptio placentae.

### *Previous Investigations*

In the present section reference will be made only to publications in which the analyses, in premature babies, have been based

### Discussion

*Taylor et al* (1963) found high excretion values of oestriol in pregnancies complicated only by Rh-immunisation. If this is correct it would be necessary in such cases to raise the upper limit of the oestriol output supposed to indicate that the foetus is stressed. However both the present series and that of *Klopper and Stephenson* (1966) show that the oestriol excretion in such cases is within the range found in completely normal pregnancies, and consequently the limits of the "danger zone" need no alterations.

### SUMMARY

The excretion of oestriol in 156 cases of apparent Rh-immunisation was found to be in the same range as that of normal pregnancies

### REFERENCES

- Banerjee S* J Obst. Gyn. Br Com. 69 963 1962  
*Coyle M, and Brown J B* J Obst. Gyn. Br Com. 70 225 1963  
*Frandsen V* Ugeskr Læg 24 1231 1962  
*Frandsen V and Stakemann G* Acta Endocr 44 183 1963  
*Frandsen V and Stakemann G* Dan. Med. Bull. 7 95 1960  
*Klopper A. and Stephenson R.* J Obst. Gyn. Br Com. 73 282, 1966  
*Schindler A. Ratonasopa V Lee T and Herrmann W* Obst. Gyn. 29 625, 1967  
*Taylor S Hassner A. Bruns P and Drose V* Am. J Obst. Gyn. 85 10 1963

Received on July 18 1968



## COAGULATION AND FIBRINOLYSIS IN PREMATURELY DELIVERED MOTHERS AND THEIR PREMATURE INFANTS

by

NIELS CHL. NIELSEN

In a number of recent communications it has been confirmed that the content of several coagulation factors in the blood increases as pregnancy advances. Fibrinolysis during pregnancy has also been thoroughly studied.

Coagulation and fibrinolysis in premature babies have been studied in a number of publications. However it is difficult to compare the studies, because the blood samples were taken at different times after delivery and because different techniques were used. Moreover the term prematurity is usually based only upon the birth weight (under 2500 g) without paying regard to gestational age.

The object of the present work was to study coagulation and fibrinolysis in a well-defined group of infants born more than four weeks before term in whom the blood samples were removed under standardized conditions. The results will be compared with those obtained by analyses on maternal blood.

This material allows subsequent comparison with similar findings in premature babies from pregnancies complicated by e.g. to rhesus iso-immunization, pre-eclampsia and abruptio placentae.

### *Previous Investigations*

In the present section reference will be made only to publications in which the analyses, in premature babies, have been based

upon cord blood, since a number of changes occur very soon after birth in coagulation (Beller 1955 Vest and Meier 1957 Hansen 1960 and others) as well as in fibrinolysis (Engström and Kager 1964)

### *First Phase of Coagulation.*

**Platelets** In two comprehensive studies Talbert and Langdell (1964) and Nilsson and Kullander (1967) found that the maternal platelet count did not change during the last trimester

In blood from the umbilical vein Hansen (1960) found sub-normal platelet counts average 168 000/mm<sup>3</sup>, in a study of 47 premature infants but he did not state any control values from mature infants.

**Factor VIII** (antihaemophilic factor AHF) The reported findings have varied somewhat but recent investigations have shown the content to increase through pregnancy (Strauss and Diamond 1963 Kasper et al. 1964 Nilsson and Kullander 1967 and others)

Factor VIII activity is reduced in the cord blood of premature but not in mature infants. Kün er (1959) for instance, found an average of only 60 per cent of normal although a number of the values were in the normal range

**Factor IX** (Christmas factor) Rarnoff and Holland 1959 and Kasper et al 1964 have reported an increased level in the blood in the 3rd trimester of pregnancy while Nilsson and Kullander (1967) and others were unable to demonstrate such an increase.

Kün er (1959) has demonstrated a marked reduction of factor IX in the cord blood of all premature babies so studied.

The prolonged recalcification time reported by Kün er (1959) is in keeping with the reduced content of factors VIII and IX.

### *Second Phase of Coagulation*

**Factor II** (prothrombin) Beller (1957) and Fresh et al (1959) found an increase in maternal blood during the latter part of pregnancy while Kasper et al (1964) and Talbert and Langdell (1964) found only slight or no changes

Table I. Results of Coagulation and Fibrinolysis Studies in Normal Women Immediately Delivery

Tests, see Methods	Normal Women Immediately after Delivery (Weight of Newborns)					
	>2500 g		>2000 g <2500 g		≤2000	
	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD
Platelets	207	67-351	231	139-448	215	172-257
(Thousands per cu. mm.)	n=20	±76	n=10	±90	n=10	±27
Prothrombin	110	80-190	101	75-125	110	87-134
(per cent)	n=20	±31	n=10	±17	n=10	±14
Prothrombin time	17	16-20	18	15-20	18	17-20
(seconds)	n=20	±0.9	n=10	±1.7	n=10	±0.8
P and P	206	120-380	178	115-245	166	140-210
(per cent)	n=20	±68	n=10	±44	n=10	±23
Partial thromboplastin	73	66-79	75	67-85	75	71-81
time (seconds)	n=20	±3.6	n=10	±5	n=10	±3.2
Thrombin time	8	5-10	8	5-8	7	5-8
(seconds)	n=20	±1.1	n=10	±1	n=10	±1.2
Fibrinogen	508	320-985	515	440-650	519	390-730
(mg per 100 ml)	n=20	±154	n=10	±57	n=10	±107
Haematocrit	42	38-48	41	35-55	42	38-46
(per cent)	n=20	±3.2	n=10	±1.8	n=10	±2.7
Standard fibrin plates	0	0	0	0	0	0
Untreated plasma						
(sq. mm.)	n=20	±0	n=10	±0	n=10	±0
Standard fibrin plates	39	0-105	30	0-93	21	0-49
Engelbohm (sq. mm.)	n=20	±25	n=10	±35	n=10	±16
Heated fibrin plates	0	0	0	0	0	0
Untreated plasma						
(sq. mm.)	n=20	±0	n=10	±0	n=10	±0
Heated fibrin plates	27	0-49	21	0-40	14	4-49
Engelbohm (sq. mm.)	n=20	±14	n=10	±14	n=10	±14
Plasminogen (mg Cu-	173	103-234	169	126-194	175	125-223
Tyrosine per ml.)	n=20	±25	n=10	±18	n=10	±28

 $\bar{x}$ —arithmetic average, n—number of estimations

Range—range of individual results ( ) est. SD—estimated standard deviation

$$= \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

In previous studies on the relation of prothrombin levels to the maturity of the baby some authors have found lower and others higher values in premature than in mature infants but invariably the concentration has been reduced compared with adults. For reviews of the earlier literature the reader is referred to *Dyggve* 1952 and *Larsen* 1952. A large series, submitted by *Forgács et al.* (1962) who studied cord blood from 100 premature infants of birth weights from 750 to 1500 g compared with 104 full term infants clearly showed that the prothrombin content is lowest in the former group. No details are given concerning the composition of this group.

**Factor VII (proconvertin)** *Alexander et al.* (1956) *Beller* (1957) *Pechet* and *Alexander* (1961) and others have reported an increase in the maternal blood in the last trimester.

In cord blood the proconvertin level is reduced (*Künzer* 1959 *Hansen* 1960 and *Forgács* 1962). The last-mentioned team also demonstrated lower values in premature than in mature infants.

**Factor X (Stuart Prower factor)** : An increased content has been demonstrated in the maternal blood during the last trimester (*Pechet* and *Alexander* 1961) while in the cord blood of premature babies it has been found to be reduced (*Künzer* and *Ströder* 1960).

**Factor V (proaccelerin)** A number of workers have found an unchanged factor V level throughout pregnancy (including *Alexander et al.* 1956 *Kasper et al.* 1964 *Talbert* and *Langdell* 1964 and *Nilsson* and *Kullander* 1967).

According to some reports factor V too is reduced in the blood of premature babies (*Künzer* 1959 and *Forgács et al.* 1962) while *Hansen* (1960) found a level above the normal range but he himself doubted whether this result was correct.

### *Third Phase of Coagulation*

**Factor I (fibrinogen)** The blood level steadily increases throughout pregnancy (*Fresh et al.* (1959) *Gillman et al.* (1959) *Kasper et al.* (1964) *Talbert* and *Langdell* (1964) *Brakman* (1966) *Nilsson* and *Kullander* (1967) and others). However

Table II. Results of Coagulation and Fibrinolysis Studies in the Newborn

Tests, see Methods	Infants Immediately after Delivery					
	Weight					
	>2500 g		>2000 g<2500 g		≤2000 g	
	x and n	Range est. SD	x and n	Range est. SD	x and n	Range est. SD
Platelets (thousands per cu. mm.)	323	56-571	307	174-625	294	200-384
	n=23	±147	n=10	±144	n=10	±63
Prothrombin (per cent)	115	62-170	92	58-115	85	34-150
	n=20	±34	n=10	±21	n=10	±32
Fibrinogen time (seconds)	19	17-27	19	17-23	2	20-24
	n=20	±1.5	n=10	±2.1	n=10	±2.3
P and P (per cent)	72	30-115			38	23-56
	n=20	±25			n=10	±11
P and P' (per cent)	90	62-190	44	27-78		
	n=20	±21	n=10	±16		
Partial thromboplastin time (seconds)	88	78-125	112	97-144	142	104-212
	n=20	±11	n=10	±15	n=10	±32
Thrombin time (seconds)	13	10-15	10	7-14	14	9-24
	n=20	±1.5	n=10	±2.3	n=10	±4.7
Fibrinogen (mg per 100 ml)	242	150-340	264	140-370	221	145-270
	n=20	±50	n=10	±67	n=10	±60
Hematocrit (per cent)	55	48-63	56	47-65	56	49-69
	n=20	±4.9	n=10	±5.6	n=10	±4.0
Standard fibrin plates Untreated plasma (sq mm.)	86	18-230	38	0-54	51	25-70
	n=20	±56	n=10	±22	n=10	±20
Standard fibrin plates Erythrocytes (sq mm.)	390	251-658	211	25-394	210	56-360
	n=20	±119	n=10	±104	n=10	±100
Heated fibrin plates Untreated plasma (sq mm.)	25	9-49	12	0-25	11	0-20
	n=20	±9.7	n=10	±8.3	n=10	±6.2
Heated fibrin plates Erythrocytes (sq mm.)	34	19-49	27	1-49	24	16-36
	n=20	±7.6	n=10	±12	n=10	±5.7
Plasminogen (mg Co- Tymanox per ml)	67	37-108	33	17-46	37	8-65
	n=20	±19	n=10	±12	n=10	±17

See footnote to Table I

Prophylactic treatment of the mothers with vitamin K.

In previous studies on the relation of prothrombin levels to the maturity of the baby some authors have found lower and others higher values in premature than in mature infants, but invariably the concentration has been reduced compared with adults. For reviews of the earlier literature the reader is referred to *Dyggve* 1952 and *Larsen* 1952. A large series submitted by *Forgács et al* (1962) who studied cord blood from 100 premature infants of birth weights from 750 to 1500 g compared with 104 full term infants clearly showed that the prothrombin content is lowest in the former group. No details are given concerning the composition of this group.

**Factor VII** (proconvertin) - *Alexander et al* (1956) *Beller* (1957) *Pechet* and *Alexander* (1961) and others have reported an increase in the maternal blood in the last trimester.

In cord blood the proconvertin level is reduced (*Künzler* 1959 *Hansen* 1960 and *Forgács* 1962). The last-mentioned team also demonstrated lower values in premature than in mature infants.

**Factor X** (Stuart Prower factor) An increased content has been demonstrated in the maternal blood during the last trimester (*Pechet* and *Alexander* 1961) while in the cord blood of premature babies it has been found to be reduced (*Künzler* and *Ströder* 1960).

**Factor V** (proaccelerin) A number of workers have found an unchanged factor V level throughout pregnancy (including *Alexander et al* 1956 *Kasper et al* 1964 *Talbert* and *Langdell* 1964 and *Nilsson* and *Kullander* 1967).

According to some reports factor V too is reduced in the blood of premature babies (*Künzler* 1959 and *Forgács et al* 1962) while *Hansen* (1960) found a level above the normal range but he himself doubted whether this result was correct.

### *Third Phase of Coagulation*

**Factor I** (fibrinogen) The blood level steadily increases throughout pregnancy (*Fresh et al* (1959) *Gillman et al* (1959) *Kasper et al* (1964) *Talbert* and *Langdell* (1964) *Brakman* (1966), *Nilsson* and *Kullander* (1967) and others). However

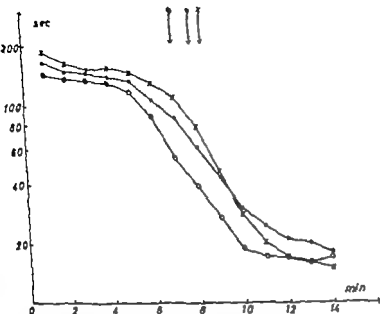


Fig 1 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 20 normal women at term.

- 15 normal adult women, two-stage TAT
- 20 normal women, two-stage TAT immediately after delivery
- 20 normal women, three-stage TAT immediately after delivery

or on serum or else the groups of babies studied were not directly comparable.

A reduced fibrinolytic activity in premature as compared with mature infants has been demonstrated by Arustowicz (1966) and Markarian *et al.* (1967) who reported a reduced fibrinolytic activity in euglobulin plasma, by Künzler and Stroder (1961) and Ambrus *et al.* (1963) largely on the basis of an increased antipainin level in the premature babies. On the other hand, Forgács *et al.* (1962) found the fibrinolytic activity to be in the same range in premature and in mature infants. Lastly Challa and Lurachi (1954) reported that fibrinolytic activity was most marked in premature and stillborn children, but perhaps this result was due to anoxia.

Phillips and Skrodellis (1958) found the values to be the same in women during labour whether at or before term.

Forgács *et al* (1962) found a lower fibrinogen content in the cord blood of premature than of mature infants. Phillips and Skrodellis (1958) and Panizza (1962) arrived at the same result, but with such a marked range of values that there can hardly have been significant differences. On the other hand, Arustowicz (1966) observed a significantly lower content in premature babies. However her technique of drawing blood was not ideal. The blood was drawn directly from the cord where it must have been in contact with the gelatinous substance with its high content of thromboplastin. Markarian *et al*. (1967) demonstrated a tendency to a higher fibrinogen content in premature babies, but six hours after birth there was practically no difference.

*Haematocrit* Most investigations have shown slightly decreasing haematocrit values towards term but with an increase shortly before. (For review of the literature cf Nielsen 1963)

The haematocrit level in cord blood from premature infants does not appear to have been investigated.

### *Fibrinolysis*

Several studies have shown that fibrinolytic activity is reduced during the last months of pregnancy while it is normal or increased immediately after delivery. The literature has been reviewed by Nielsen (1963) and by Nilsson and Kullander (1967) who also reported steadily increasing plasminogen content during pregnancy. In delivered women at term Samart'is *et al*. (1960) Skjodt and Albrechtsen (1965) and others have also found an increased plasminogen activity whereas Shaper *et al*. (1965) and Brakman (1966) were unable to demonstrate any difference in the content between non-pregnant and pregnant women. Comparison of women in labour at term and women delivering healthy premature infants has shown no difference in fibrinolytic activity (Markarian *et al* (1967))

The reported data on fibrinolytic activity in the cord blood of premature babies have varied. This may be due to different techniques the analyses may have been performed on plasma



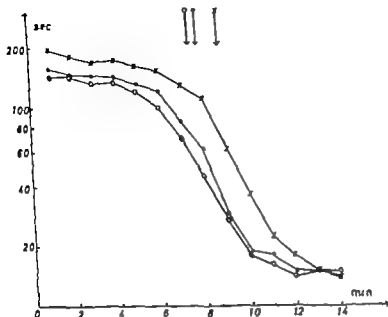


Fig 3 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of six weeks before estimated date of confinement

- 9 normal adult women two-stage TAT
- 10 women, two-stage TAT immediately after delivery
- 10 women, three-stage TAT immediately after delivery

delivery and puerperium had otherwise been normal. The samples were removed within the first half-hour after delivery. Prior to delivery all the women (except one whose analysis did not differ from the others) had received prophylactic vitamin K, in a dosage as described previously (Nielsen 1969 a).

Blood samples were also drawn from these women's newborn infants within the first 20 minutes after birth. All the infants were healthy premature babies who behaved normally during their stay in hospital. The birth weights were below 2500 g but above 2000 g, average 2270 g (range 2020–2440 g).

Group 2 Ten women and their newborn infants selected according to the same criteria as group 1 only with delivery ap-

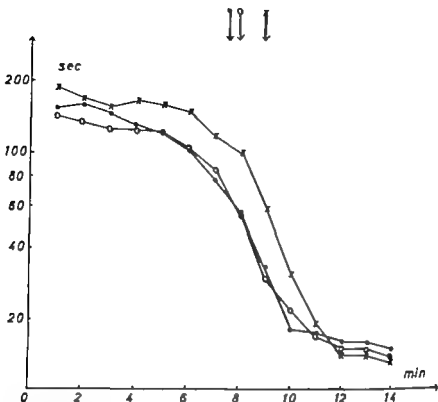


Fig. 2. Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of five weeks before estimated date of confinement.

- x — x 10 normal adult women two-stage TAT  
 ● — ● 10 women two-stage TAT immediately after delivery  
 ○ — ○ 10 women three-stage TAT immediately after delivery

Plasminogen activity on the other hand is reduced in the newborn particularly when they are premature (Phillips and Skrodelis 1958 Qule and Wannamaker 1960 Samartzis *et al.* 1960 Paul *a* 1962 and Ambrus *et al.* 1963)

#### Material and Methods

The analyses were performed on blood samples from the following three groups

Group 1 Ten women who were delivered about 5 weeks (range 4–7 weeks) before estimated term but whose pregnancy

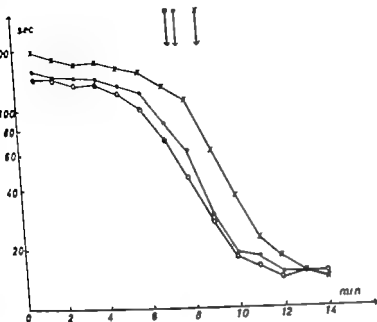


Fig 3 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of six weeks before estimated date of confinement.

- 9 normal adult women, two-stage TAT
- 10 women, two-stage TAT immediately after delivery
- 10 women, three-stage TAT immediately after delivery

delivery and puerperium had otherwise been normal. The samples were removed within the first half-hour after delivery. Prior to delivery all the women (except one whose analysis did not differ from the others) had received prophylactic vitamin K, in a dosage as described previously (Nielsen 1969 a).

Blood samples were also drawn from these women's newborn infants within the first 20 minutes after birth. All the infants were healthy premature babies who behaved normally during their stay in hospital. The birth weights were below 2500 g, but above 2000 g, average 2270 g (range 2020–2440 g).

Group 2 Ten women and their newborn infants selected according to the same criteria as group 1 only with delivery ap-

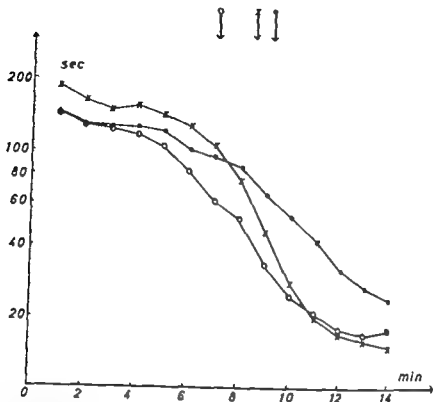


Fig. 4 Thromboplastin activation curve of venous cord blood from 20 normal mature infants.

- x — x 15 normal adult women, two-stage TAT
- — ● 20 normal mature infants two-stage TAT
- — ○ 20 normal mature infants three-stage TAT

prox. 6 weeks before estimated term (range 4–8 weeks) and birth weight  $\leq 2000$  g, average 1780 g (range 1440–2000 g). One infant, apparently normal at birth, died on the second day of life. Autopsy revealed total pulmonary atelectasis with congestion and incipient formation of hyaline membranes. The women in this group had not received prophylactic vitamin K.

**Group 3** The control series comprised two groups. One of 20 normal women delivered at term and their normal newborn infants. Vitamin K was administered and blood samples drawn just as in group 1. The other group also contained 20 normal women delivered at term and their normal newborn infants, but

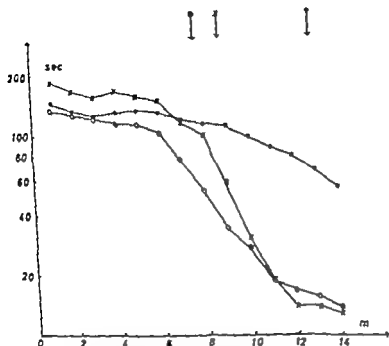


Fig. 5. Thromboplastin activation curve of venous cord blood from 10 normal premature infants, weights between 2500 g and 2000 g.

- 10 normal adult women, two-stage TAT
- — 10 normal premature infants, two-stage TAT
- 10 normal premature infants three-stage TAT

In this group no vitamin K was administered. This control series has previously been described (Nielsen 1969 a).

The maternal blood samples were removed from an ante-cubital vein after application of light tourniquet. In the newborn the umbilical cord was clamped close to the skin and a disposable plastic catheter was inserted through the umbilical vein. The samples were used only if there was free flow of blood through the catheter. The technique of blood sampling and of the further treatment of the samples has been described in detail in previous publications. Determination of the recalcification time, thromboplastin activation test (TAT) two- and three-stage, partial thromboplastin time (PTT) thrombin time prothrombin time (Quick) factor V prothrombin-proconvertin (PP) fibrinogen content, and measurement of fibrinolytic activity in plasma and in iso-electrically precipitated plasma on untreated fibrin

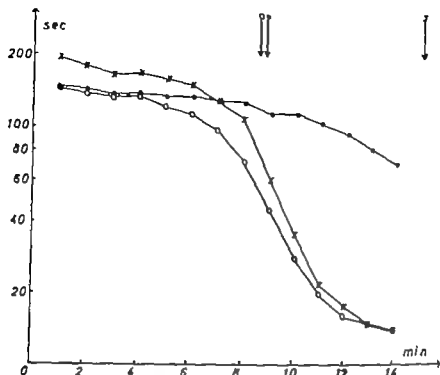


Fig. 6 Thromboplastin activation curve of venous cord blood from 10 normal premature infants weight below 2001 g.

- x — x 9 normal adult women, two-stage TAT  
 ● — ● 10 normal premature infants two-stage TAT  
 ○ — ○ 10 normal premature infants three-stage TAT

plates (standard plates) and on heated fibrin plates as well as plasminogen, haematocrit, and platelet counts were also performed as already described (Nielsen 1969 a)

### Results

The results of the *coagulation studies* are presented in Tables I and II those of TAT and recalcification times in Figs. 1 2, 3 4 5 and 6 For that part of the control group in which the mothers received prophylactic vitamin K all the results have been omitted with the exception of the babies PP since this is the only factor which is definitely influenced by vitamin K (Nielsen 1969 a)

In a comparison between group 1 (mothers with premature infants weighing between 2000 and 2500 g) and group 3 (nor-

al mothers and their infants) the following findings were made

A. *Peripheral maternal blood.* TAT two-stage showed no major differences between the two groups except that  $t_{\max}$  was significantly shortened in the prematurely delivered women ( $0.02 > p > 0.01$ ). TAT three-stage showed coagulation-activating components in the plasma from both groups possibly somewhat less in the prematurely delivered women.

Apart from the shortened thrombin time ( $p < 0.001$ ) in prematurely delivered women, there were no significant differences in coagulation findings.

B. *Cord blood.* TAT two-stage showed a distinctly flatter course in the premature babies with a significantly prolonged recalcification time ( $p < 0.001$ ) and  $t_{\max}$  ( $0.01 > p > 0.001$ ). TAT three-stage was practically identical in both groups. The individual coagulation analyses demonstrate a considerably lower level in premature babies. This applies for instance to PP ( $p < 0.01$ ) and PTT ( $p < 0.001$ ). The thrombin time was shortened ( $p < 0.001$ ) whereas no change was found in fibrinogen content.

The results of the fibrinolysis studies are also listed in Tables I and II. A comparison of group 1 with group 3 shows the following findings:

A. *Peripheral maternal blood.* No significant difference between the two groups.

B. *Cord blood.* Significantly reduced fibrinolytic activity in premature babies, in untreated plasma on standard plates ( $0.01 > p > 0.001$ ) and on heated fibrin plates ( $0.01 > p > 0.001$ ) as well as in iso-electrically precipitated plasma on standard plates ( $p < 0.001$ ). The plasminogen content too was reduced in the premature babies ( $p < 0.001$ ).

In order to elucidate the influence of the degree of prematurity upon coagulation and fibrinolysis, group 1 (average birth weight 2270 g) was compared with group 2 (average birth weight 1780 g). The results were as follows:

A. *Peripheral maternal blood.* No significant changes in the parameters of coagulation or fibrinolysis.

B. *Cord blood.* Within the group of infants whose birth weight averaged 1780 g there was a distinctly flatter curve for TAT

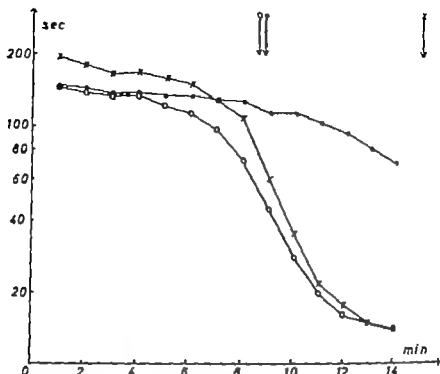


Fig. 6. Thromboplastin activation curve of venous cord blood from 10 normal premature infants, weight below 2001 g

- x—x 9 normal adult women two-stage TAT  
 ●—● 10 normal premature infants two-stage TAT  
 ○—○ 10 normal premature infants three-stage TAT

plates (standard plates) and on heated fibrin plates, as well as plasminogen, haematocrit, and platelet counts were also performed as already described (Nielsen 1969a)

### Results

The results of the *coagulation studies* are presented in Tables I and II those of TAT and recalcification times in Figs. 1 2, 3 4 5 and 6. For that part of the control group in which the mothers received prophylactic vitamin K all the results have been omitted with the exception of the babies PP since this is the only factor which is definitely influenced by vitamin K (Nielsen 1969a)

In a comparison between group 1 (mothers with premature infants weighing between 2000 and 2500 g) and group 3 (nor-



mal mothers and their infants) the following findings were made

A. *Peripheral maternal blood* TAT two-stage showed no major differences between the two groups except that  $t$  max was significantly shortened in the prematurely delivered women ( $0.02 > p > 0.01$ ) TAT three-stage showed coagulation-activating components in the plasma from both groups, possibly somewhat less in the prematurely delivered women.

Apart from the shortened thrombin time ( $p < 0.001$ ) in prematurely delivered women, there were no significant differences in coagulation findings.

B. *Cord blood* TAT two-stage showed a distinctly flatter course in the premature babies with a significantly prolonged recalcification time ( $p < 0.001$ ) and  $t$  max ( $0.01 > p > 0.001$ ) TAT three-stage was practically identical in both groups. The individual coagulation analyses demonstrate a considerably lower level in premature babies. This applies for instance to PP ( $p < 0.01$ ) and PTT ( $p < 0.001$ ) The thrombin time was shortened ( $p < 0.001$ ) whereas no change was found in fibrinogen content

The results of the fibrinolysis studies are also listed in Tables I and II. A comparison of group 1 with group 3 shows the following findings

A. *Peripheral maternal blood*. No significant difference between the two groups

B. *Cord blood*. Significantly reduced fibrinolytic activity in premature babies, in untreated plasma on standard plates ( $0.01 > p > 0.001$ ) and on heated fibrin plates ( $0.01 > p > 0.001$ ) as well as in iso-electrically precipitated plasma on standard plates ( $p < 0.001$ ) The plasminogen content too was reduced in the premature babies ( $p < 0.001$ )

In order to elucidate the influence of the degree of prematurity upon coagulation and fibrinolysis, group 1 (average birth weight 2270 g) was compared with group 2 (average birth weight 1780 g) The results were as follows

A. *Peripheral maternal blood* No significant changes in the parameters of coagulation or fibrinolysis.

B. *Cord blood* Within the group of infants whose birth weight averaged 1780 g there was a distinctly flatter curve for TAT

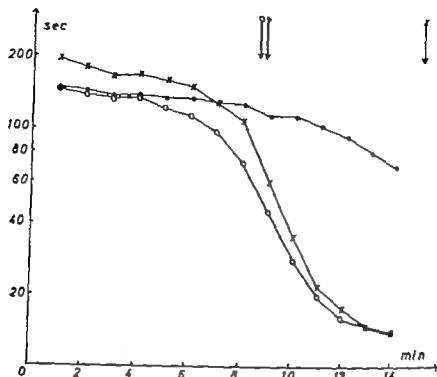


Fig. 6 Thromboplastin activation curve of venous cord blood from 10 normal premature infants weight below 2001 g.

- x — x 9 normal adult women, two-stage TAT  
 • — • 10 normal premature infants two-stage TAT  
 o — o 10 normal premature infants three-stage TAT

plates (standard plates) and on heated fibrin plates, as well as plasminogen, haematocrit, and platelet counts were also performed as already described (Nielsen 1969 a)

### Results

The results of the *coagulation studies* are presented in Tables I and II, those of TAT and recalcification times in Figs. 1, 2, 3, 4, 5 and 6. For that part of the control group in which the mothers received prophylactic vitamin K all the results have been omitted with the exception of the babies PP since this is the only factor which is definitely influenced by vitamin K (Nielsen 1969 a)

In a comparison between group 1 (mothers with premature infants weighing between 2000 and 2500 g) and group 3 (nor-

with increasing prematurity. Accordingly the results correspond with those in *Forgård et al.* (1962) comprehensive study and appear to be in keeping with most of the findings reported in the literature.

*Third phase of coagulation.* As is apparent from the submitted literature, the fibrinogen content increases as pregnancy advances. In this material, too, there was an increased content in women after delivery but this increase was of the same magnitude in women who were delivered at term as in those who went into labour about 6 weeks before the estimated date of confinement. Thus, the maximum fibrinogen content appears to be attained at a fairly early stage of the third trimester. The thrombin time was also the same in all three groups of delivered women.

No difference was found in the content of fibrinogen in premature and mature infants, but the results are comparable to those already published. However the results for premature babies in the study of *Forgård et al.* (1962) were lower but as already mentioned their material consisted of infants who were more premature than in the present series.

Indeed, the thrombin time was the same for mature infants and for those having a birth weight averaging 1780 g.

In all three groups of mothers (at term, about 5 weeks and about 6 weeks before term) the TAT three-stage showed coagulation-activating components of approximately the same magnitude. The same applies to the three groups of infants. This supports the theory that it is the mechanism of delivery which is the provoking factor. For a more detailed discussion of these findings, cf. *Nielsen* (1969 a, 1969 b).

The reduced content of coagulation factors II, V, VII, and X in the premature infants may be partly explained as a consequence of their immature liver function, and partly because of consumption, induced by coagulation-activating components in the plasma. The same explanation may apply to the reduction of factors VIII and IX whose origin is unknown.

If fibrinogen were synthesized together with the other factors in the liver it would also have been expected to be reduced, but no such reduction was demonstrable.

two-stage with a significantly prolonged recalcification time ( $0.05 > p > 0.02$ ) while the TAT three-stage curve showed no major changes. PTT was significantly prolonged ( $0.02 > p > 0.01$ ) and the thrombin time was again at the same level as for normal infants born at term. Otherwise there were no significant changes in coagulation or fibrinolysis.

### *Discussion*

*First phase of coagulation.* The present investigations confirm previous reports that the platelet count does not alter through the last trimester of pregnancy. On the basis of the methods used, which are screening tests it is established that the content of the factors which make up the first phase of coagulation is increased in prematurely delivered women as well as in women who are delivered at term—*cf* the PTT and TAT two-stage, including the recalcification time. However it is not possible to decide neither from the literature nor from the present results whether this applies to factor VIII as well as factor IX or merely to one of them.

The platelet count in the cord blood did not differ significantly in the three groups of newborn infants. However there is a tendency to lower values in the premature groups. This is in keeping with the results of Hansen (1960) although the absolute figures are not comparable. For factors VIII and IX, there is presumably a question of marked reduction of both most marked in the most premature group.

*Second phase of coagulation.* In conformity with other workers an evenly increasing PP has been demonstrated throughout the last months of pregnancy. The negligible differences in the Quick times are presumably due to the unchanged content of factor V throughout pregnancy. This too accords with previous reports.

In the newborn the present semiquantitative analytical methods could demonstrate a decreasing content of the factors in the second phase of coagulation (*viz* factors II V VII and X) with increasing prematurity. This is further confirmed by the increasing flattening of the TAT two-stage curve which occurs

with increasing prematurity. Accordingly the results correspond with those in *Forgdcs et al.* (1962) comprehensive study and appear to be in keeping with most of the findings reported in the literature.

*Third phase of coagulation.* As is apparent from the submitted literature, the fibrinogen content increases as pregnancy advances. In this material, too, there was an increased content in women after delivery but this increase was of the same magnitude in women who were delivered at term as in those who went into labour about 6 weeks before the estimated date of confinement. Thus, the maximum fibrinogen content appears to be attained at a fairly early stage of the third trimester. The thrombin time was also the same in all three groups of delivered women.

No difference was found in the content of fibrinogen in premature and mature infants, but the results are comparable to those already published. However the results for premature babies in the study of *Forgdcs et al.* (1962) were lower but as already mentioned their material consisted of infants who were more premature than in the present series.

Indeed, the thrombin time was the same for mature infants and for those having a birth weight averaging 1780 g.

In all three groups of mothers (at term, about 5 weeks and about 11 weeks before term) the TAT three-stage showed coagulation-activating components of approximately the same magnitude. The same applies to the three groups of infants. This supports the theory that it is the mechanism of delivery which is the provoking factor. For a more detailed discussion of these findings, cf. *Nielsen* (1969 a, 1969 b).

The reduced content of coagulation factors II, V, VII and X in the premature infants may be partly explained as a consequence of their immature liver function, and partly because of consumption, induced by coagulation-activating components in the plasma. The same explanation may apply to the reduction of factors VIII and IX whose origin is unknown.

If fibrinogen were synthesized together with the other factors in the liver it would also have been expected to be reduced, but no such reduction was demonstrable.

### Fibrinolysis

The present studies of fibrinolysis showed no significant difference in fibrinolytic activity or in plasminogen content between mothers who went into labour prematurely and mothers who went to term. This agrees with the findings during delivery (*Markarian et al* 1967). The results presented here of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on standard plates were the same for mothers at term as those reported by *Nilsson and Kullander* (1967).

In accordance with a number of previous reports, a reduced fibrinolytic activity was found in the cord blood of premature babies, in unprecipitated plasma as well as in the euglobulin fraction. These observations differ from those of *Forgács et al.* (1962) who found a normal fibrinolytic activity in premature babies. The explanation may be that their series included very small infants, weighing 750 g. These infants may have been hypoxic when the blood samples were drawn, and in that event fibrinolytic activity is increased.

The low plasminogen content in the premature babies is in accordance with the results from the literature. The explanation must be poorly developed hepatic function rather than auto-digestion caused by spontaneous activation during delivery as *Quie and Wannamaker* (1960) have demonstrated that this reduction persists for at least 6 weeks after birth.

A reduced fibrinolytic activity and a low plasminogen content may according to *Ambrus et al.* (1963) involve a danger of hyaline membrane formation. As already mentioned their investigation showed a marked reduction of fibrinolytic activity in premature babies, and particularly low values in infants having hyaline membrane disease. It is assumed that the reduced activity may prevent the dissolution of fibrin which is the basic element of the hyaline membrane. On the other hand, *Lieberman and Kellogg* (1961) believe that hyaline membrane formation is due to the presence of an activator inhibitor in the lungs.

## SUMMARY

In a study of coagulation and fibrinolysis conditions in prematurely delivered women and their premature infants the author made the following findings, compared with normal women delivered at term and their full-time infants

*A. Maternal findings.* A tendency to a prolonged recalcification time and a somewhat lower PP per cent in the prematurely delivered women. In both groups coagulation-activating components were found in the plasma.

Otherwise, no differences in the coagulation or fibrinolysis conditions. No difference in haematocrit.

*B Findings in the newborn.* A highly prolonged recalcification time and a greatly reduced PP per cent plus a highly prolonged PTT in the premature babies. On the other hand, no changes in platelet count or fibrinogen content. The changes were most marked in the most premature infants.

In mature as well as in premature infants the TAT three-stage revealed coagulation-activating components in the plasma.

A distinct reduction in fibrinolytic activity and a reduction in plasminogen level was demonstrated in the premature babies. No difference in the haematocrit was found.

## REFERENCES

- Alexander B Meyers L, Kenney J, Goldstein R, Gersmehl V and Grossman L. *New Engl. J Med.* 254 358, 1956  
Ambros C M, Weinstaub D H, Dancphy D, Dowd, J E, Pickren J W, Nussmeider K R and Ambros J L. *Pediatrics* 32 10 1963  
Anstomies Z. *Ann Paediat.* 206 265 1966  
Beller F K. *Rev belge path.* 24 422, 1955  
Das Gerinnungsverhältnisse bei der Schwangerschaft und beim Neugeborenen, J A Barth, Leipzig, 1957  
Brabner P. *Amer J Obstet Gynec* 94 11 1966  
Cifola U and Lamaschi C. *Ann Obstet Gynec.* 76 207 1954  
Dyggve H V. *Undersøgelse over K-vitaminets betydning for blodet* hos nyfødte. Nyt Nordisk Forlag, København 1952  
Egertsen L and Kager L. *Acta paedat.* 53 326, 1964  
Forsgren J, Nemeth L and Elek E. *Gynaecologia* 154 29 1962

### *Fibrinolysis.*

The present studies of fibrinolysis showed no significant difference in fibrinolytic activity or in plasminogen content between mothers who went into labour prematurely and mothers who went to term. This agrees with the findings during delivery (Markarian *et al* 1967) The results presented here of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on standard plates were the same for mothers at term as those reported by Nilsson and Kullander (1967)

In accordance with a number of previous reports, a reduced fibrinolytic activity was found in the cord blood of premature babies, in unprecipitated plasma as well as in the euglobulin fraction. These observations differ from those of Forgács *et al* (1962) who found a normal fibrinolytic activity in premature babies. The explanation may be that their series included very small infants, weighing 750 g These infants may have been hypoxic when the blood samples were drawn, and in that event fibrinolytic activity is increased.

The low plasminogen content in the premature babies is in accordance with the results from the literature. The explanation must be poorly developed hepatic function rather than auto-digestion caused by spontaneous activation during delivery as Quie and Wannamaker (1960) have demonstrated that this reduction persists for a least 6 weeks after birth.

A reduced fibrinolytic activity and a low plasminogen content may according to Ambrus *et al* (1963) involve a danger of hyaline membrane formation. As already mentioned, their investigation showed a marked reduction of fibrinolytic activity in premature babies, and particularly low values in infants having hyaline membrane disease It is assumed that the reduced activity may prevent the dissolution of fibrin which is the basic element of the hyaline membrane On the other hand, Lieberman and Kellogg (1961) believe that hyaline membrane formation is due to the presence of an activator inhibitor in the lungs.



## INFLUENCE OF PRE ECLAMPSIA UPON COAGULATION AND FIBRINOLYSIS IN WOMEN AND THEIR NEWBORN INFANTS IMMEDIATELY AFTER DELIVERY

By

NIELS CHR. NIELSEN

Intravascular haemolysis and changes in coagulation and fibrinolysis have long been known as complications of eclampsia. Alterations in the parameters of coagulation and fibrinolysis have also been reported in pre-eclampsia, but so far only case reports or studies of individual factors have been published. There do not seem to be any studies on the possible influence of pre-eclampsia upon coagulation and fibrinolysis in the newborn apart from a single paper by Villalba Trujinos (1967) on the fibrinogen content.

Assessment of the available literature causes considerable difficulties, because very often the composition of the series is not clearly defined. Accordingly the results are frequently based upon cases of pre-eclampsia as well as patients with chronic hypertension, chronic pyelonephritis, chronic glomerulonephritis, etc. etc.

The object of the present study is to throw some light upon coagulation and fibrinolysis in a well-defined group of mothers with pre-eclampsia and their newborn infants.

### *Previous Investigations*

In the review of the literature the author has included as far as possible only the results of studies on patients who fulfil the same criteria of pre-eclampsia as those in the present series (cf. *Material and Methods*). It was tried also to exclude cases of eclampsia.

- Fresh J W Adams H and Morgan F M, *Obstet. and Gynec.* 13 37 1959  
Gillman T Naidoo S S and Hathorn M. *Lancet* 2 70 1959  
Hansen H G., *Z. Kinderheilk.* 84 327 1960  
Kasper C K. Hoag M. S Aggeler P M. and Stone S. *Obstet. and Gynec.* 24 242, 1964  
Künzer W *Machr Kinderheilk.* 107 110 1959  
Künzer W and Ströder J. *Ann. paediat.* 195 137 1960  
- *Ibidem* 197 9 1961  
Larsen J Svingainger I prothrombinaaktiviteten hos nyfødte, E. Munksgaards forlag, København, 1952  
Lieberman J and Kellogg, F. *Calif. Med.* 95 278, 1961  
Markerian M. Githens J H Jackson J J Bannon A. E., Lindley A Rosenblat E. Martorell R. and Lubcheno L. O. *Amer J Dis. Child.* 113 312, 1967  
Nielsen N C. *Acta obst. et gynec. scandinav* 48 371 1969 a  
- *Ibidem* 48 392 1969 b  
Nielsen P A. *Acta obstet. gynec. scand.* 42 suppl 2 1963  
Nilsson I M and Kullander S. *Acta obst. et gynec. scandinav* 46 273 1967  
Panizza G. *Acta paediat.* 1st 15 678 1962  
Pechet L. and Alexander B. *New Engl. J. Med.* 265 1093 1961  
Phillips L L and Skrodellis V. *Pediatrics* 22 715 1958  
Quie P G and Wannamaker L. W. *Amer J Dis. Child.* 100 836, 1960  
Ratnoff O D and Holland T R. *Ann. N Y Acad. Sci.* 75 626 1959  
Samaritis E. A. Cook C D and Rudolph A. J. *Acta paediat.* 49 727 1960  
Shaper A. G Macintosh D M. Evans C. M. and Kyobe J. *Lancet* 2 706, 1963  
Skjodt P and Albrechtsen O K. *Acta obst. et scandinav* 44 416, 1965  
Strauss H S. and Diamond L. R. *New Engl. J. Med.* 269 1251 1963  
Talbert L. M. and Langdell R. D. *Amer J Obstet. Gynec.* 90 44 1964  
Vest M. and Merier W. *Ann. paediat.* 189 282 1957

Received on Oct 7 1968

To my knowledge there have been no studies of the platelet count in the newborn infants of pre-eclamptic mothers, and there do not seem to have been any studies made of the other factors involved in the first phase of coagulation either in mothers or infants.

### *Second Phase of Coagulation*

Little has been published concerning the second-phase coagulation factors. Stefani and Dameshek (1955) stated that hypoprothrombinaemia was often present in pre-eclampsia, but without further elaborating on the subject. On the other hand, Szirmai (1956) on the background of a study by Arvey and Szirmai (1951) established that in toxæmia the blood level of prothrombin was higher than in normal pregnant women. However no accurate data are given concerning the results or the composition of the series. In respect of factor VII (proconvertin) there seems to be only one report, by Ciulla (1955) who stated that the values were higher for pre-eclamptics than for normal pregnant women, but without producing further evidence.

There do not appear to have been any studies of these factors on the newborn infants of pre-eclamptic mothers.

### *Third Phase of Coagulation*

**Fibrinogen.** The fibrinogen content in the blood of women with pre-eclampsia has been adequately investigated. A number of results are listed in Table I. These results are based upon a number of different techniques which are not mentioned, as it is not the absolute values, but the difference between normal pregnant women and pre-eclamptics which is of interest. The exact composition of the groups of patients is often unknown, as it is not stated by Vars Kishore *et al.* or by McHary and Corey while Mack *et al.* Dieckmann Alvarez and Afonso and Searls use, in broad terms, the definition of pre-eclampsia of The American Committee on Maternal Welfare.

As may be seen from Table I, there is hardly any significant difference in fibrinogen content between normal and pre-eclamptic women, before during or after labour.

### *First Phase of Coagulation*

**Platelets** In a study of 10 patients having moderate toxæmia diagnosed on the basis of moderate hypertension, albuminuria, and varying degrees of oedema" Ward and MacArthur (1948) demonstrated an average platelet count of 155 000/mm<sup>3</sup>, i.e. in the same range as in a control series of normal pregnant women. On the other hand, there was reduction in the platelet count of "six severe pre-eclamptic and one eclamptic this group showed a higher elevation of blood pressure and increase in the amount of albuminuria, and a marked degree of oedema the mean value being stated as 97 000 Dieckmann (1952) who used the criteria of pre-eclampsia set up by The American Committee on Maternal Welfare with minor modifications reported no difference in platelet counts between normal pregnant women and pregnant women with pre-eclampsia. His report is based upon only a few examinations and the exact values are not mentioned. Prichard *et al.* (1954) found no thrombocytopenia in 13 pre-eclamptic patients the criterion being fixed as a platelet count below 100 000/mm<sup>3</sup>. However in some of their patients they observed a fall from approx. 150 000 to 110 000–120 000 followed by an increase to 200 000 or over after delivery. These authors do not state their diagnostic criteria. Ferguson (1956) studying 16 pre-eclamptics found no major difference in the platelet count as compared with normal pregnant women. Pre-eclampsia, they state, was defined as "persistent hypertension (140/90) or albuminuria that first appeared after the twenty-fourth week of pregnancy and disappeared soon after delivery. The same result was reported by Szlinnyal *et al.* (1962) in a series of 20 pre-eclamptic patients but the basis for the diagnosis is not stated, and by Zielinska and Solecka (1962) in 21 pregnant patients with slight toxæmia (blood pressure 130/90–170/100). In five patients with severe toxæmia (blood pressure 170/100–200/100) however the latter authors found a significantly reduced platelet count. Brain *et al.* (1967) have published a case of haemolysis and thrombocytopenia in a pre-eclamptic (diagnosed in the 24th week) and tabulated a number of previous case reports on severe pre-eclampsia accompanied by thrombocytopenia.

Fibrinogen in cord blood was studied by Villalba Triviño (1967) who found, in 22 newborn infants of mothers with clinical toxæmia only half the mean value measured in 60 children of normal mothers. He gives no explanation of the massive reduction in the infants of toxæmic mothers.

For further elucidation of the third phase of coagulation there have been a few reports on thrombin time. Pritchard *et al.* (1954) found a prolonged thrombin time in 10 out of 13 patients with pre-eclampsia. Szirmay *et al.* (1962) found no difference in thrombin time between normal and toxæmic pregnant women. Searle (1966) comparing 37 patients with mild pre-eclamptic toxæmia with 24 normal pregnant women, concluded that the thrombin time tended to be prolonged in the pre-eclamptic group. This conclusion is based upon the following mean thrombin times: control group 9.12 ( $\pm 0.211$ ) sec. test group 9.46 ( $\pm 0.904$ ) sec. However, as already mentioned, only the last mentioned report was based upon a well-defined group of patients.

To my knowledge there have been no reports on thrombin times in the newborn infants of pre-eclamptic mothers.

**Haematocrit.** It has been pointed out by Berlin *et al.* (1952) and Dieckmann (1952) that pregnant women with severe pre-eclampsia have haemoconcentration. In mild pre-eclampsia, on the other hand, there does not seem to be any difference in the haematocrit as compared with normal pregnancy (Dieckmann 1952). Chowdhury (1961) found no difference in a study of normal and pre-eclamptic mothers (without further substantiating the diagnosis). On the other hand, the haematocrit in the cord blood of the newborn infants of pre-eclamptics was a more recent study. Henger (1967) comparing 19 pre-eclamptics (defined as at least 2 of the 3 symptoms: blood pressure above 140/90, proteinuria exceeding 0.5 g/l, and oedema) and 20 normal pregnant women, found no difference in the haematocrit.

**Fibrinolysis.** There seems to have been no systematic study of the fibrinolytic system in a well-defined group of patients with pre-eclampsia or their newborn infants. Smith and Smith (1945) and Smith (1947) assumed that in toxæmia hormone production

Table L. Plasma Fibrinogen Content During Late Pregnancy Delivery and Puerperium (mg/100 ml)

Authors	Diagnosis	Results		Time
		$\bar{x}$ and n	Range SD	
Mach et al. (1951)	normal pregnancy	610 17	320-840	delivery
	mild pre-eclampsia	640 8	490-890	delivery
	severe pre-eclampsia	670 9	440-1010	delivery
Dieckmann (1952)	normal pregnancy	480	300-700	at term
	pre-eclampsia	510	300-900	at term
Vera (1958)	normal delivery	578 34		immediately post partum
	mild pre-eclampsia	514 23		immediately post partum
Kishore et al (1962)	normal pregnancy	551 53	420-694 $\pm 53$	immediately post partum
	pre-eclampsia	599 8	531-683 $\pm 57$	immediately post partum
Alvarez and Afonso (1964)	normal pregnancy	390 17	$\pm 80$	1st-6th day post partum
	mild pre-eclampsia	410 20	$\pm 120$	1st-6th day post partum
	severe pre-eclampsia	442 19	$\pm 150$	1st-6th day post partum
	normal pregnancy	522 6	412-655	1st day post partum
	pre-eclampsia	547 11	434-680	post partum
Searle (1966)	normal pregnancy	400 24	$\pm 47$	late pregnancy
	mild pre-eclampsia	500 37	$\pm 80$	late pregnancy

$\bar{x}$  = arithmetic average n = number of estimations  
 range = range of individual results SD = standard deviation

Table III. Results of Coagulation and Fibrinolysis Studies in Newborn Infants

Tests see Methods	Normal Infants		Infants of Pre-Eclamptic Mothers		Premature Infants	
	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD
Platelets (thousands per cu mm.)	323 n=20	86-571 $\pm 147$	217 n=10	108-321 $\pm 68$	307 n=10	174-625 $\pm 144$
Prothrombin (per cent)	115 n=20	62-170 $\pm 34$	91 n=10	70-120 $\pm 17$	92 n=10	59-115 $\pm 21$
Prothrombin time (seconds)	110 n=20	17-22 $\pm 1.5$	21 n=10	19-25 $\pm 1.8$	19 n=10	17-23 $\pm 2.1$
P and P (per cent)	72 n=20	30-115 $\pm 25$	46 n=10	34-79 $\pm 13$		
and P <sup>a</sup> (per cent)	90 n=20	62-150 $\pm 21$			44 n=10	27-78 $\pm 16$
Partial thromboplastin time (seconds)	■ n=20	78-125 $\pm 11$	108 n=10	83-128 $\pm 18$	112 n=10	97-144 $\pm 15$
Thrombin time (seconds)	13 n=20	10-15 $\pm 1.5$	19 n=10	11-31 $\pm 7.0$	10 n=10	7-14 $\pm 2.3$
Fibrinogen (mg per 100 ml)	242 n=20	150-340 $\pm 50$	177 n=10	95-250 $\pm 59$	264 n=10	140-370 $\pm 67$
Hematocrit (per cent)	55 n=20	46-63 $\pm 4.9$	57 n=10	50-62 $\pm 3.7$	56 n=10	47-65 $\pm 5.8$
Standard fibrin plates Untreated plasmas (sq mm.)	88 n=20	16-230 $\pm 56$	49 n=10	0-121 $\pm 44$	38 n=10	0-54 $\pm 22$
Standard fibrin plates Euglobulins (sq mm.)	390 n=20	251-658 $\pm 119$	251 n=10	0-589 $\pm 192$	211 n=10	25-324 $\pm 104$
Heated fibrin plates Untreated plasmas (sq mm.)	25 n=20	9-49 $\pm 9.7$	19 n=10	0-36 $\pm 14$	12 n=10	0-25 $\pm 8.3$
Heated fibrin plates Euglobulins (sq mm.)	34 n=20	19-49 $\pm 7.0$	24 n=10	0-40 $\pm 13$	27 n=10	1-49 $\pm 12$
Fibrinogen (mg Co- roline per ml)	67 n=20	37-108 19	55 n=10	19-102 $\pm 28$	33 n=10	17-46 $\pm 12$

See footnote to Table II

Prophylactic treatment of the mothers with vitamin K

Table II. Results of Coagulation and Fibrinolysis Studies in Normal and Pre-Eclamptic Women Immediately after Delivery

Tests see Methods	Normal Women at Term		Pre-Eclamptic Women 2-3 Weeks Before Term		Normal Women 5 Weeks Before Term	
	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD.
Platelets (thousands per cu. mm.)	207 n=20	67-351 ±76	152 n=10	113-188 ±26	231 n=10	139-448 ±90
Proaccelerin (per cent)	110 n=20	80-190 ±31	118 n=10	102-145 ±15	101 n=10	15-125 ±17
Prothrombin time (seconds)	17 n=20	16-20 ±0.9	17 n=10	16-19 ±0.9	18 n=10	15-20 ±1.7
P and P (per cent)	206 n=20	120-380 ±68	190 n=10	162-225 ±17	18 n=10	115-245 ±44
Partial thromboplastin time (seconds)	73 n=20	66-79 ±3.6	77 n=10	71-83 ±3.6	75 n=10	67-83 ±5.0
Thrombin time (seconds)	8 n=20	5-10 ±1.1	7 n=10	5-8 ±1.1	6 n=10	5-8 ±1.0
Fibrinogen (mg per 100 ml)	508 n=20	320-965 ±144	466 n=10	340-595 ±82	515 n=10	440-650 ±57
Haematocrit (per cent)	42 n=20	36-48 ±3.2	41 n=10	34-47 ±4.7	41 n=10	35-55 ±1.8
Standard fibrin plates Untreated plasma (sq mm.)	0 n=20	0 ±0	0 n=10	0 ±0	0 n=10	0 ±0
Standard fibrin plates Euglobulins (sq mm.)	39 n=20	0-125 ±25	28 n=10	0-49 ±15	30 n=10	0-93 ±35
Heated fibrin plates Untreated plasma (sq mm.)	0 n=20	0 ±0	0 n=10	0 ±0	0 n=10	0 ±0
Heated fibrin plates Euglobulins (sq mm.)	27 n=20	0-49 ±14	26 n=10	4-45 ±14	21 n=10	0-40 ±14
Plasminogen (μg Cu- Tyrosine per ml.)	173 n=20	108-234 ±25	161 n=10	130-214 ±26	169 n=10	126-199 ±18

$\bar{x}$  = arithmetic average n = number of estimations  
range = range of individual results (x) est. SD = estimated standard deviation

$$= \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$



One patient went into labour spontaneously and had a normal delivery 3 had a normal delivery after the membranes had been ruptured, 3 had forceps deliveries, and 3 had Caesarean sections (2 after the onset of labour and 1 elective) In 5 cases prophylactic vitamin K was administered prior to delivery in doses as described previously (Nielsen 1969 a) Delivery occurred on average 2 or 3 weeks before term (range 0-6 weeks) The blood samples were drawn within the first half-hour after delivery Moreover blood samples were removed from these patients newborn infants within the first 20 minutes after birth Birth weight averaged 2690 g (range 1500-4150 g) During the stay in the ward the infants showed no abnormalities, apart from the signs of prematurity

**Group 2** The control series comprised, firstly a group of 20 normal delivered women at term and their normal newborn infants these women did not receive prophylactic vitamin K Also, a group of 10 women treated prophylactically with vitamin K, delivered about 5 weeks (range 4-7 weeks) before calculated term, but having an otherwise normal pregnancy delivery and puerperium, and their premature infants (mean birth weight 2270 g (range 2070-2400 g)) These control series have been described previously (Nielsen 1969 a 1969 b)

The maternal blood samples were drawn from an ante-cubital vein after the application of a light tourniquet. In the newborn the umbilical cord was incised close to the skin, and a disposable plastic catheter was inserted through the umbilical vein The samples were used only if there was free flow of blood through the catheter The technique of the blood sampling and the further treatment of the samples have been described previously Determination of the recalcification time, the thromboplastin activation test (TAT) two- and three-stage, partial thromboplastin time (PTT) thrombin time prothrombin time (Quick) factor V prothrombin-proconvertin (PP) fibrinogen content, and measurement of fibrinolytic activity in plasma and in iso-electrically precipitated plasma on untreated fibrin plates (standard plates) and on heated fibrin plates as well as plasminogen, haematocrit, and platelet counts were also performed as described previously (Nielsen 1969 )

### Results

The results of the coagulation studies are given in Tables II and III those of the TAT and recalcification times in Figs 1 2, 3 4 5

was reduced and that this resulted in increased catabolism and the formation of a "toxin" supposed to activate coagulation and increase fibrinolytic activity. In accordance with this theory they found an increased fibrinolytic activity in euglobulin serum from 11 pre-eclamptic or eclamptic patients in the second and third trimester of pregnancy on comparison with 6 normal pregnant women. However these results must be considered with reserve as the patients were in the second or third trimester i.e. some of them at a stage of pregnancy when the diagnosis of pre-eclampsia cannot normally be accepted. Moreover the series includes an unknown number of eclamptic patients.

Willson and Munnel (1946) found no fibrinolytic activity in the peripheral blood of normal pregnant women while fibrinolysis occurred in chronic vascular-renal diseases associated with proteinuria and oedema as well as in pre-eclampsia. More recent studies by S Innayi *et al.* (1962) and Searle (1966) have shown no difference in fibrinolytic activity between normal and pre-eclamptic pregnant women, but their reports too are based upon very rough screening of the fibrinolytic system.

### *Material and Methods*

The analyses were performed on blood samples from the following two groups

*Group 1* Ten women (9 primiparae and 1 para-2) with pre-eclampsia selected according to the following criteria (a) healthy before the pregnancy (b) normal blood pressure i.e. in no case exceeding 130 mm Hg systolic or 90 mm Hg diastolic during the first two trimesters of pregnancy (c) increasing blood pressure during the third trimester calling in all cases for treatment with pethidine-chlorpromazine or Stroganoff (mean blood pressure on medication at time of delivery 180/110 mm Hg (range 160/90-205/120 mm Hg) (d) simultaneously with the increase in blood pressure oedema and proteinuria [at the time of delivery a mean of 0.5 per cent (range 0.04-1.4 per cent)] (e) blood pressure returned to normal and proteinuria subsided after delivery so that at follow-up about 8 weeks after delivery the patient had normal blood pressure and no proteinuria.

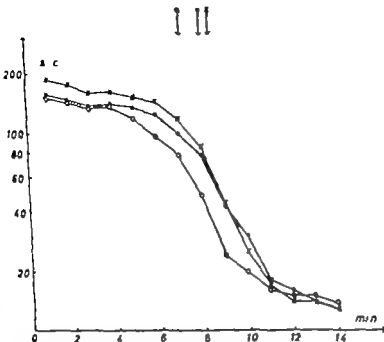


Fig. 2. Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women with pre-eclampsia.

- 10 normal adult women, two-stage TAT
- 10 women with pre-eclampsia, two-stage TAT immediately after delivery
- △— 10 women with pre-eclampsia, three-stage TAT immediately after delivery

no major differences between the two groups. However  $t_{max}$  was shortened in pre-eclamptic mothers ( $0.05 > p > 0.02$ ). By TAT three-stage, coagulation-activating components in the same range were found in the plasma from both groups.

II *Cord blood* In the newborn infants of pre-eclamptic mothers the coagulation factors were considerably reduced. This was manifested by a prolonged Quick time ( $0.01 > p > 0.001$ ) a reduced PP level ( $p < 0.001$ ) factor V level ( $0.05 > p > 0.02$ ) fibrinogen ( $0.01 > p > 0.001$ ) and platelet count ( $0.02 > p > 0.01$ ) simultaneously with a prolonged PTT ( $0.01 > p > 0.001$ ) and

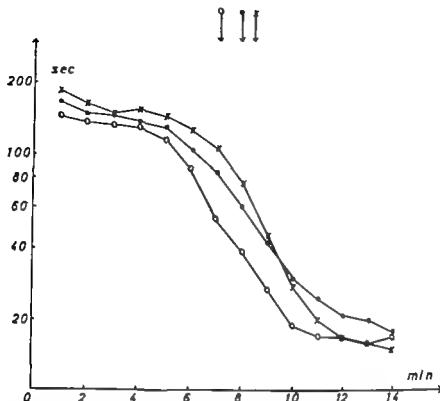


Fig. 1 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 20 normal women at term.

x — x 15 normal adult women, two-stage TAT

● — ● 20 normal women two-stage TAT immediately after delivery

○ — ○ 20 normal women, three-stage TAT immediately after delivery

and 6 Since the PP level in the newborns is the only parameter of coagulation fibrinolysis which is susceptible to the administration of vitamin K to the mothers (Nielsen 1969 a) these values, for the newborn of untreated as well as treated mothers, are given in Table III.

Comparison of normal mothers at term and their newborn infants with pre-eclamptic mothers and their newborn infants revealed the following significant differences

A. *Peripheral maternal blood* A reduced platelet count in the pre-eclamptic mothers ( $0.01 > p > 0.001$ ) and a prolonged PTT ( $0.01 > p > 0.001$ ) In addition, a shortened thrombin time in the pre-eclamptics ( $0.01 > p > 0.001$ ) TAT two-stage showed

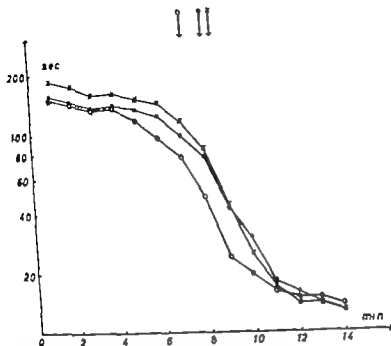


Fig. 2 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women with pre-eclampsia.

- x 10 normal adult women, two-stage TAT
- — 10 women with pre-eclampsia, two-stage TAT immediately after delivery
- 10 women with pre-eclampsia, three-stage TAT immediately after delivery

no major differences between the two groups. However,  $t_{max}$  was shortened in pre-eclamptic mothers ( $0.05 > p > 0.02$ ). By TAT three-stage, coagulation-activating components in the same range were found in the plasma from both groups.

**B. Cord blood** In the newborn infants of pre-eclamptic mothers the coagulation factors were considerably reduced. This was manifested by a prolonged Quick time ( $0.01 > p > 0.001$ ), a reduced PP level ( $p < 0.001$ ), factor V level ( $0.05 > p > 0.02$ ), fibrinogen ( $0.01 > p > 0.001$ ) and platelet count ( $0.02 > p > 0.01$ ) simultaneously with a prolonged PTT ( $0.01 > p > 0.001$ ) and

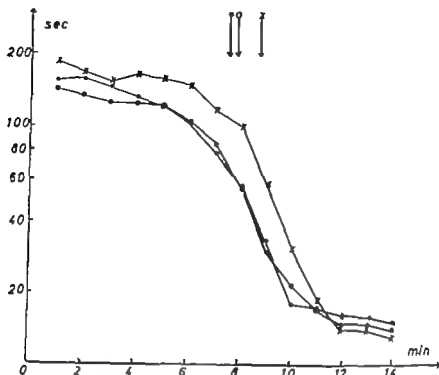


Fig 3 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of five weeks before estimated date of confinement.

x — x 10 normal adult women, two-stage TAT

● — 10 women two-stage TAT immediately after delivery

○ — 10 women, three-stage TAT immediately after delivery

thrombin time ( $0.01 > p > 0.001$ ) In accordance with these findings the TAT two-stage curve was flatter than the corresponding curve for normal infants. At the same time the recalcification time was prolonged but not significantly from 565 sec. to 620. By the TAT three-stage coagulation activating components were demonstrated in the plasma from both groups, possibly more marked in the newborn infants of pre-eclamptic mothers.

The results of the *fibrinolysis studies* are also given in Tables II and III. A comparison of the same groups showed

A *Peripheral maternal blood* No significant difference

II *Cord blood* Significantly reduced fibrinolytic activity in the plasma euglobulin fraction in the newborn infants of pre-

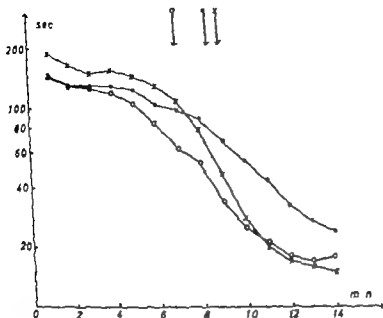


Fig. 4 Thromboplastin activation curve of venous cord blood from 20 normal newborn infants

- x 15 normal adult women, two-stage TAT
- o 20 normal infants, two-stage TAT
- Δ 20 normal infants, three-stage TAT

eclamptic mothers upon determinations on standard fibrin plates ( $0.05 > p > 0.02$ ) as well as on heated fibrin plates ( $0.05 > p > 0.02$ )

Since the duration of pregnancy influences the factors concerned with coagulation and fibrinolysis, Tables II and III also give the results for a group of mothers delivered approximately 5 weeks before term and their premature infants, selected as described under "Material and Methods"

Comparison of the group of pre-eclamptic mothers, who were delivered 2 or 3 weeks before term, with a group of mothers who were delivered approximately 5 weeks before term showed that the above-mentioned differences in platelet count cannot be explained as a result of the duration of pregnancy since the platelet counts in the pre-eclamptics are also significantly lower than

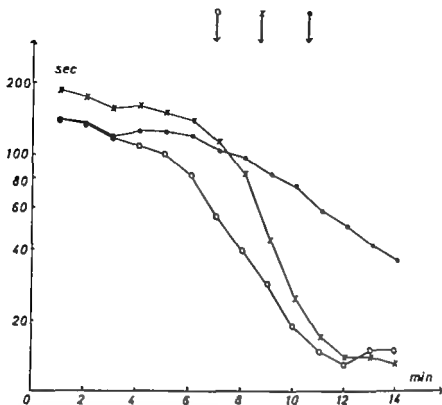


Fig. 5. Thromboplastin activation curve of venous cord blood from 10 newborn infants of pre-eclamptic mothers.

x — x 10 normal adult women, two-stage TAT

● — ● 10 infants of pre-eclamptic mothers two-stage TAT

○ — ○ 10 infants of pre-eclamptic mothers three-stage TAT

those of the prematurely delivered, otherwise normal women ( $0.05 > p > 0.02$ ). On the other hand, this comparison showed no significant differences in PTT or  $t_{\max}$  and the thrombin time of the pre-eclamptics was midway between the values for women delivered at term and prematurely. TAT two- or three-stage showed no major differences between pre-eclamptics and prematurely delivered women. However according to the TAT three-stage there seems to be a tendency to a more pronounced activation in the pre-eclamptics.

Newborn infants of pre-eclamptic mothers have a prolonged thrombin time ( $0.01 > p > 0.001$ ) and a reduced fibrinogen content ( $0.01 > p > 0.001$ ) whereas other factors are in the same



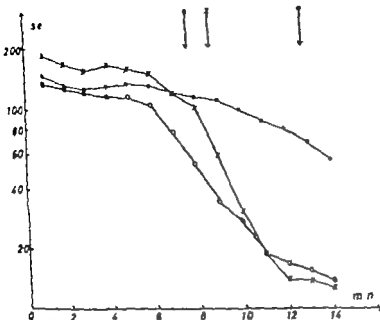


Fig 6 Thromboplastin activation curve of venous cord blood from 10 premature newborn infants

- x — 10 normal adult women, two-stage TAT
- 10 premature infants two-stage TAT
- — 10 premature infants, three-stage TAT

range as in premature infants of normal mothers. The TAT two-stage curve was not quite as flat in the infants of pre-eclamptic mothers as in the otherwise normal premature infants and the recalcification time was shorter but not significantly 620 sec. as compared with 770 sec. According to the TAT three-stage there seems to be a somewhat more pronounced coagulation activity in the plasma of the newborn infants of pre-eclamptic mothers than in the premature infants.

Fibrinolytic activity in the newborn infants of pre-eclamptic mothers and in premature infants proved to be in the same range only the content of plasminogen was significantly reduced in the premature group ( $0.05 > p > 0.02$ )

### Discussion

The present results do not indicate any major difference in the factors concerned with coagulation and fibrinolysis between normal mothers and pre-eclamptic mothers. This is confirmed by a study (Skjodr personal communication) on the parameters of coagulation and fibrinolysis in pre-eclamptics before, during, and after delivery.

The relatively low platelet count in the pre-eclamptics is possibly explained by consumption due to a slightly increased activation of the coagulation system. This idea is supported by the report of McKay *et al.* (1964) who demonstrated increased platelet adhesiveness in pre-eclamptic patients, interpreted as a sign of a "lower grade process of disseminated intravascular coagulation in pre-eclampsia".

The reported results concerning platelet counts in pre-eclamptics are not consistent perhaps because of the heterogeneous groups studied. However the platelet count seems to be reduced in severe forms of pre-eclampsia a tendency confirmed by the present investigations.

The presence of mild intravascular coagulation may also explain the prolonged PTT and the slightly reduced fibrinogen level in pre-eclamptics.

Other parameters of coagulation and fibrinolysis proved to lie, as expected, between those for mothers delivered at term and mothers delivered approx. 5 weeks before term. A prolonged thrombin time or changes in fibrinolysis could not be demonstrated.

The infants of pre-eclamptic mothers on the other hand, show considerable changes in the parameters of coagulation. These changes may be interpreted as the consequences of a degree of intravascular coagulation, as manifest by a reduction in the platelet count of the PP factor V and fibrinogen levels, as well as in a prolongation of PTT Quick time and thrombin time. The course of the TAT three-stage curve is also compatible with an increased content of coagulation activating components in the plasma.

The reduced content of coagulation factors in the infants of

pre-eclamptic mothers cannot be explained by birth at an average of 2 or 3 weeks before term, as the values were in several cases even lower than in infants born, on average 5 weeks prematurely to normal mothers.

Fibrinolysis does not seem to undergo major changes apart from those which may be ascribed to slight prematurity.

A number of other publications have supported the assumption of slight intravascular coagulation in pre-eclamptic mothers. This applies to McKay and Corey (1964) on the background of their study of cryofibrinogen, and to Maurner *et al.* (1962) Beller (1964) and others who have demonstrated subendothelial depositions of fibrin in the capillary coils of the renal glomeruli.

As pointed out by McKay *et al.* (1964) it must be emphasized that intravascular coagulation in eclampsia is different from that in pre-eclampsia. In eclampsia there is a sudden and massive process leading to capillary thrombosis, visible in the light microscope. In pre-eclampsia, on the other hand coagulation is slower and incomplete, so that capillary occlusion does not occur but, as already mentioned, there is a deposition of fibrin on the basement membrane of the renal glomeruli.

The cause of this intravascular coagulation is uncertain. Schneider (1951, 1952) suggested liberation of thromboplastin from a retroplacental or intradecidual haematoma or tissue thromboplastin of placental origin. As stated by McKay *et al.* (1964) the cause may also be coagulation-activating components released from platelets which have been destroyed by adhering to the syncytial trophoblast which is damaged for some unknown reason.

If one of these theories is correct, there ought to be a possibility of intravascular coagulation also in the newborn infant. Indeed, Leissring and Vorlicky (1968) have recently published a case in which coagulation studies and later autopsy of the newborn infant of a pre-eclamptic mother showed massive intravascular coagulation.

### SUMMARY

In a study of coagulation and fibrinolysis in pre-eclamptic mothers and their newborn infants, comparison with normal women de-

### Discussion

The present results do not indicate any major difference in the factors concerned with coagulation and fibrinolysis between normal mothers and pre-eclamptic mothers. This is confirmed by a study (Skjoldt personal communication) on the parameters of coagulation and fibrinolysis in pre-eclampsics before, during, and after delivery.

The relatively low platelet count in the pre-eclampsics is possibly explained by consumption due to a slightly increased activation of the coagulation system. This idea is supported by the report of McKay *et al* (1964) who demonstrated increased platelet adhesiveness in pre-eclamptic patients interpreted as a sign of a lower grade process of disseminated intravascular coagulation in pre-eclampsia.

The reported results concerning platelet counts in pre-eclampsics are not consistent perhaps because of the heterogeneous groups studied. However the platelet count seems to be reduced in severe forms of pre-eclampsia a tendency confirmed by the present investigations.

The presence of mild intravascular coagulation may also explain the prolonged PTT and the slightly reduced fibrinogen level in pre-eclampsics.

Other parameters of coagulation and fibrinolysis proved to lie as expected, between those for mothers delivered at term and mothers delivered approx. 5 weeks before term. A prolonged thrombin time or changes in fibrinolysis could not be demonstrated.

The infants of pre-eclamptic mothers on the other hand, show considerable changes in the parameters of coagulation. These changes may be interpreted as the consequences of a degree of intravascular coagulation, as manifest by a reduction in the platelet count of the PP factor V and fibrinogen levels as well as in a prolongation of PTT Quick time and thrombin time. The course of the TAT three-stage curve is also compatible with an increased content of coagulation-activating components in the plasma.

The reduced content of coagulation factors in the infants of

pre-eclamptic mothers cannot be explained by birth at an average of 2 or 3 weeks before term, as the values were in several cases even lower than in infants born, on average 5 weeks prematurely to normal mothers.

Fibrinolysis does not seem to undergo major changes apart from those which may be ascribed to slight prematurity.

A number of other publications have supported the assumption of slight intravascular coagulation in pre-eclamptic mothers. This applies to McKay and Corey (1964) on the background of their study of cryofibrinogen, and to Maurner *et al.* (1962) Beller (1964) and others who have demonstrated subendothelial depositions of fibrin in the capillary coils of the renal glomeruli.

As pointed out by McKay *et al.* (1964) it must be emphasized that intravascular coagulation in eclampsia is different from that in pre-eclampsia. In eclampsia there is a sudden and massive process leading to capillary thrombosis, visible in the light microscope. In pre-eclampsia, on the other hand coagulation is slower and incomplete so that capillary occlusion does not occur but, as already mentioned, there is a deposition of fibrin on the basement membrane of the renal glomeruli.

The cause of this intravascular coagulation is uncertain. Schneider (1951 1952) suggested liberation of thromboplastin from a retroplacental or intradecidual haematoma or tissue thromboplastin of placental origin. As stated by McKay *et al.* (1964) the cause may also be coagulation-activating components released from platelets which have been destroyed by adhering to the syncytial trophoblast which is damaged for some unknown reason.

If one of these theories is correct, there ought to be a possibility of intra-vascular coagulation also in the newborn infant. Indeed, Leissring and Vorlicek (1968) have recently published a case in which coagulation studies and later autopsy of the newborn infant of a pre-eclamptic mother showed massive intra-vascular coagulation.

## SUMMARY

In a study of coagulation and fibrinolysis in pre-eclamptic mothers and their newborn infants, comparison with normal women de-

### Discussion

The present results do not indicate any major difference in the factors concerned with coagulation and fibrinolysis between normal mothers and pre-eclamptic mothers. This is confirmed by a study (Skjodt personal communication) on the parameters of coagulation and fibrinolysis in pre-eclampsics before, during, and after delivery.

The relatively low platelet count in the pre-eclampsics is possibly explained by consumption due to a slightly increased activation of the coagulation system. This idea is supported by the report of McHay *et al* (1964) who demonstrated increased platelet adhesiveness in pre-eclamptic patients interpreted as a sign of a "lower grade process of disseminated intravascular coagulation in pre-eclampsia".

The reported results concerning platelet counts in pre-eclampsics are not consistent perhaps because of the heterogeneous groups studied. However the platelet count seems to be reduced in severe forms of pre-eclampsia a tendency confirmed by the present investigations.

The presence of mild intravascular coagulation may also explain the prolonged PTT and the slightly reduced fibrinogen level in pre-eclampsics.

Other parameters of coagulation and fibrinolysis proved to lie as expected, between those for mothers delivered at term and mothers delivered approx. 5 weeks before term. A prolonged thrombin time or changes in fibrinolysis could not be demonstrated.

The infants of pre-eclamptic mothers on the other hand show considerable changes in the parameters of coagulation. These changes may be interpreted as the consequences of a degree of intravascular coagulation, as manifest by a reduction in the platelet count, of the PP factor V and fibrinogen levels as well as in a prolongation of PTT Quick time and thrombin time. The course of the TAT three-stage curve is also compatible with an increased content of coagulation activating components in the plasma.

The reduced content of coagulation factors in the infants of

- Nilner, N. C., *Acta obst. gynec. scandinav.* 48, 37 1969 a
- Ibidem* 48 505 1969 b
- Prichard, J. A., Ratnoff O. D. and Wetsman R., *Obstet. and Gynec.* 4 159 1954
- Schander C. L. J. *Obstet. Gynaec. Brit. Emp.* 58 538 1951
- Azner, J. *Obstet. Gynec.* 63 1078 1952
- Swale J. W. *Amer. N. Z. J. Obstet. Gynaec.* 6 142, 1966
- Smith, O. W. and Smith, G. V. *Science* 102 253, 1945
- Smith O. W. *Amer. J. Obstet. Gynec.* 54 201 1947
- Stefenat M. and Dameshek, W. *The Hemorrhagic Disorders*, Grune & Stratton, New York, 1955
- Szmydy, M. Császár S. and Káplár Z. *Zbl. Gynäk.* 84 460 1962
- Sztröm, E. *Gynaecologia* 141 359 1956
- Vera, P. *Geburtsk. u. Frauenheilk.* 18 432 1958
- Villalón Trillo N. C., *Acta pediat. exp.* 25 273, 1967
- Ward, C. V. and MacArthur J. L., *Amer. J. Obstet. Gynec.* 55 600 1948
- Wilson J. R. and Munroe E. R., *Proc. Soc. exp. Biol.* 62, 277 1946
- Zimbleke, C. and Solecka, W. *Ginek. pol.* 33 4 1962

Received on Oct. 7 1968

livered at term and their normal infants showed

A. *In the mothers.* Reduced platelet count prolonged PTT and a slightly reduced fibrinogen content. These results are compatible with mild intravascular coagulation.

Otherwise no differences in the parameters of coagulation or fibrinolysis and no difference in haematocrit could be shown.

B. *In the newborns.* In the infants of pre-eclamptic mothers a considerable reduction in platelet count, PP factor V and fibrinogen content as well as prolonged PTT Quick and thrombin time. The TAT three-stage showed a slightly increased content of coagulation-activating components in the plasma. These results might indicate that some degree of intravascular coagulation had taken place.

There were no changes in fibrinolytic activity apart from what may be due to the slight prematurity. There was no difference in the haematocrit.

#### REFERENCES

- Alvarez R. R. and Afonso J. F. *Amer J Obstet Gynec.* 88 774 1964  
 Arany S. and Szirmai E., *Magy Nőorv Lap* 16 277 1951 cit. after Szirmai, E. *Gynaecologia* 141 359 1956  
 Beller F. cit. after McKay D. G. De Bacalao E. B. and Sedlis A. *Amer J Obstet Gynec.* 90 1315 1964  
 Berlin N. I. Hyde G. M. Lawrence J. H. Parsons R. J. and Port S. *Surg Gynec. Obstet.* 94 71 1952  
 Bain M. C. Kaul K. B. and Dixon H. G. *J Obstet. Gynaec. Brit. Comm.* 74 702, 1967  
 Chowdhury N. N. R. *J Obstet Gynaec. India* 12 155, 1961  
 Ciulla U. 1 International Conference on Thrombosis and Embolism Benno Schwabe & Co. Basel 1955  
 Dieckmann W. J. *The Toxemias of Pregnancy* C. V. Mosby Comp. St. Louis 1952  
 Ferguson J. H. *Amer J Obstet Gynec.* 72 1315 1956  
 Hønger P. E. *Scand J clin Lab Invest.* 19 263 1967  
 Kishore N. Aggarwal T. R. and Pathak P. *Antiseptic* 59 379 1962  
 Lelwaring, J. C. and V. Ickv L. V. *Amer J Dis Child* 115 100 1968  
 Mack H. C. Robinson A. R. Wiseman M. E. Schoeb E. J. and Macy I. G. *J clin. Invest.* 30 609 1951  
 Mautner W. Chung J. Krishnan F. and Dachs S. *Lab Invest* 11 518 1966  
 McKay D. G. and Corey A. E. *Obstet and Gynec.* 23 508 1964  
 McKay D. G. De Bacalao E. B. and Sedlis A. *Amer J Obstet Gynec.* 90 1315 1964



- Nichols N C., *Acta obst. gynec. scandinav* 48 37 1969  
 Ibidem 48 505 1969 b  
 Pritchard, J A. Rasmoff O D and Wolman R., *Obstet. and Gynec.* 4 159 1954  
 Schneider C. L. *J Obstet. Gynaec. Brit. Emp* 58 538 1951  
 Amer *J Obstet. Gynec.* 63 1078, 1952  
 Searle J W. *Amst. N. Z. J. Obstet. Gynaec.* 6 142, 1966  
 Smith O W and Smith G V. *Science* 102 253, 1945  
 Smith O W. *Amer. J. Obstet. Gynec.* 54 201 1947  
 Stefanski M. and Dameshek, W. *The Hemorrhagic Disorders*, Grune & Stratton, New York, 1955  
 Szuranyi M. Csöndör S. and Képlár Z., *Zbl. Gynäk.* 84 480 1962  
 Schmal E., *Gynecologia* 141 399 1956  
 Vana P. *Geburtsk. u. Frauenheilk.* 18 432, 1958  
 Villalba Trubian N C., *Acta pediat. exp.* 25 273, 1967  
 Ward, C. V. and Mac Arthur J L., *Amer. J. Obstet. Gynec.* 55 600 1948  
 Wilton J R. and Munzel E. R., *Proc. Soc. exp. Biol.* 62 277 1946  
 Zielenka, C. and Solucha, W. *Ginek. pol* 33 4 1962

Received on Oct. 7 1968

From the Third Department of Pathology (Prof. E. Saxén) and the Department of Basic Instruction in Zoology Faculty of Medicine University of Helsinki Finland and the Department of Gynaecology and Obstetrics (L. E. Tötterman) Central Hospital Rovaniemi Finland

## INCORPORATION OF TETRACYCLINE INTO HUMAN FOETAL BONES AFTER MATERNAL DRUG ADMINISTRATION

BY

L. ERIK TÖTTERMAN<sup>1</sup> AND LAURI SAXÉN

It has long been known that tetracyclines are incorporated into growing bones (Milch *et al.* 1957 Frost *et al.* 1960) and that at high dosages they are deleterious to bone development in chick embryos (Bevelander *et al.* 1960). Clinically acceptable doses do not seem to have any morphologically demonstrable persistent effect on bone growth (Hughes *et al.* 1965). Charles (1954) demonstrated placental transmission of tetracyclines and Gibbons and Reichelderfer (1960) found that the concentration of tetracycline in cord blood sometimes exceeded the values noted in premature infants given peroral tetracycline medication. Therefore it might be expected that the administration of tetracycline during mammalian pregnancy would result in demonstrable effects on the foetal bones. Colman *et al.* (1963) showed that rat embryos like chick embryos were affected by early protracted and heavy administration of tetracycline during pregnancy. These investigators also detected tetracycline (as judged by fluorescence) in all investigated bones of a premature human foetus whose mother had been given tetracycline prior to delivery and Douglas (1963) observed fluorescence in the deciduous teeth of infants whose mothers had received tetracycline during pregnancy. However, in a controlled study of the fluore-

<sup>1</sup> Present address: Department of Basic Instruction in Zoology Faculty of Medicine University of Helsinki Finland



Fig. 1 A section of the distal phalangeal bone of the digit of a full-term infant of a mother who received tetracycline just prior to delivery (Case B Table II). Photographed in UV-light. Yellow fluorescence is seen at the newly mineralized zones of the bone.

science in the deciduous teeth after prenatal, long-term administration of tetracycline, no effect was observed when the medication had been given during the first trimester of pregnancy and fluorescence was not invariably noted with certainty even after protracted tetracycline administration during the last trimester (Porter et al. 1966). Thus considerable individual variations seem to occur in the relationship between the amount of tetracycline administered and the amount of fluorescence observed subsequently in the foetus or infant. For this reason it was thought useful to study a series of early foetuses, and some still

*From the Third Department of Pathology (Prof. E. Saxén) and the Department of Basic Instruction in Zoology Faculty of Medicine University of Helsinki Finland and the Department of Gynaecology and Obstetrics (L. E. Tötterman) Central Hospital Rovaniemi Finland*

## INCORPORATION OF TETRACYCLINE INTO HUMAN FOETAL BONES AFTER MATERNAL DRUG ADMINISTRATION

BY

L. ERIK TÖTTERMAN AND LAURI SAXÉN

It has long been known that tetracyclines are incorporated into growing bones (Milch *et al* 1957 Frost *et al* 1960) and that at high dosages they are deleterious to bone development in chick embryos (Bevelander *et al* 1960). Clinically acceptable doses do not seem to have any morphologically demonstrable persistent effect on bone growth (Hughes *et al* 1965). Charles (1954) demonstrated placental transmission of tetracyclines, and Gibbons and Reichelderfer (1960) found that the concentration of tetracycline in cord blood sometimes exceeded the values noted in premature infants given peroral tetracycline medication. Therefore it might be expected that the administration of tetracycline during mammalian pregnancy would result in demonstrable effects on the foetal bones. Cohan *et al* (1963) showed that rat embryos like chick embryos were affected by early protracted and heavy administration of tetracycline during pregnancy. These investigators also detected tetracycline (as judged by fluorescence) in all investigated bones of a premature human foetus whose mother had been given tetracycline prior to delivery and Douglas (1963) observed fluorescence in the deciduous teeth of infants whose mothers had received tetracycline during pregnancy. However in a controlled study of the fluore-



Fig 1 A section of the distal phalangeal bone of the digit of full-term infant of mother who received tetracycline just prior to delivery (Case B Table II) Photographed in UV light yellow fluorescence is seen at the newly mineralized zones of the bone.

science in the deciduous teeth after prenatal, long-term administration of tetracycline no effect was observed when the medication had been given during the first trimester of pregnancy and fluorescence was not invariably noted with certainty even after protracted tetracycline administration during the last trimester (Porter *et al.* 1966). Thus considerable individual variations seem to occur in the relationship between the amount of tetracycline administered and the amount of fluorescence observed subsequently in the foetus or infant. For this reason it was thought useful to study a series of early foetuses, and some still-

births and neonatal deaths whose mothers had received tetracycline medication during pregnancy

### *Material and methods*

During the years 1962–1966 foetuses were collected after therapeutic abortions. The main indication for termination of pregnancy was protracted physical or mental strain, but as a rule there was also some medical indication of minor importance. In 23 cases it was known that the mother had received tetracycline during pregnancy. In addition there were one full term and one premature infant who died postnatally and one premature foetus who died *in utero*.

The small foetuses with a menstrual age from 82 to 128 days (Table I) obtained by hysterotomy were stored in 10 % formalin for later investigation. Longitudinal sections of the tibia were taken for the study of fluorescence. In the older foetus and the two infants (Table II) bone tissue from the fifth toe was examined. Undecalcified samples were sectioned in paraffin and examined unstained in UV light under a Leitz Panphot microscope.

The mothers had been given one of the following tetracycline compounds: tetracycline (TC), oxytetracycline (OTC), roltetracycline (RTC). In addition tetracycline (TC) coupled to a side-chain L-methylenelysine (*de Cameri* 1961) had been administered in four cases (nos. 170, 156, 159 and 155). As appears from Tables I and II the series is not homogeneous in respect to dosage or duration or mode of administration but the time of the therapeutic termination of pregnancy at a menstrual age of about 120 days, was more or less the same in all cases except one (no. 156).

### *Results*

The microscopic observations on the presence or absence of fluorescence in the foetuses and infants examined are indicated in Tables I and II where other details of the tetracycline treatment are also given.



births and neonatal deaths whose mothers had received tetracycline medication during pregnancy

### *Material and methods*

During the years 1962-1966 foetuses were collected after therapeutic abortions. The main indication for termination of pregnancy was protracted physical or mental strain but as a rule there was also some medical indication of minor importance. In 23 cases it was known that the mother had received tetracycline during pregnancy. In addition there were one full term and one premature infant who died postnatally and one premature foetus who died *in utero*.

The small foetuses with a menstrual age from 82 to 128 days (Table I) obtained by hysterotomy were stored in 10 % formalin for later investigation. Longitudinal sections of the tibia were taken for the study of fluorescence. In the older foetus and the two infants (Table II) bone tissue from the fifth toe was examined. Undecalcified samples were sectioned in paraffin and examined unstained in UV light under a Leitz Panphot microscope.

The mothers had been given one of the following tetracycline compounds: tetracycline (TC), oxytetracycline (OTC), rolitetracycline (RTC). In addition tetracycline (TC) coupled to a side-chain L-methylenelysine (*de Cameri* 1961) had been administered in four cases (nos 170, 156, 159 and 155). As appears from Tables I and II the series is not homogeneous in respect to dosage or duration or mode of administration but the time of the therapeutic termination of pregnancy at a menstrual age of about 120 days was more or less the same in all cases except one (no 156).

### *Results*

The microscopic observations on the presence or absence of fluorescence in the foetuses and infants examined are indicated in Tables I and II where other details of the tetracycline treatment are also given.



Table 1. The Young Females Listed According to the Menstrual Age of the Last Known Period of Tetracycline Medication. Each Point in Column 3 stands for One Day of Tetracycline and the Vertical Lines Indicate the Time of the Therapeutic Abortion. The Findings in the Presence of Fluorescence Appear in Column 4

PETA #	SEX	INTERVAL TETRACYCLINE WAS ADMINISTERED	AGE AT WHICH LAST PERIOD	THIN FLUORESCENCE	CONJUGATE RIBADIL, ADMINISTRATION	TOTAL DOSE	Stage at HOME (H) or C
149	m		100	DISTINCT	TC 025-4 b TC 05-4	8	H C
	m		100		TC 05-4	4	C
165	f		100		OTC 01-2 m	0.4	C
173	m		100		TC 05-4	4	C
183	m		100	THIN	TC 025-4	3	C
168	m		100	VERY CLEAR	TC 025-4 b TC 05	6	C
154	m		100		OTC 025-4	6	H
64	f		100		a TC 025-4 b TC 05-4	8	H C
63	m		100		TC 025-4	4	H
78	m		100		TC 05-3	3	C
24			100		TC 025	4	C
109	m		100		TC 025-4	6	H
146	m		100		TC 025	4	
147	m		100	DIFFUSE	RTC 03-2	2	H
145	f		100		OTC 025-4	2	C
12	m		100		RTC 03-2	2	H
135	m		100		TC 01-2 m	0.4	C
139			100		TC 05-3	6	C
36	m		100		TC 0-2 m	0.6	C
10			100		TC 025-4		
205			100		TC 015-4	2	
70			100		RTC 03-2	3.6	
204	m		100		OTC 025-4	6	

TC-L-methylcyclopentane

Table II Stillbirths and Neonatal Deaths Menstrual Age of Tetracycline Administration (2) and of Foetal/Neonatal Death (3) Compound of Tetracycline Used (4) Amount Given per Day (5) and Total Amount (6) Fluorescence Recorded (7) and Some Clinical Comments (8)

Case	Menstrual Age at Medication	Menstrual Age at Death	Compound of TC used	Amount per Day	Total Amount (g)	Fluorescence Recorded	Clinical Comments
1	2	3	4	5	6	7	8
A	283-284	287	TC	0.25 x 4	2.5		2300 g. Placental insufficiency(?)
B	282-283	287	TC	0.5 x 6	6.0		27.0 g Anencephalus
C	185-188	2157	RTC	0.3 x 2	2.4	( )	1120 g. Infarcted placenta

### Discussion

Among the 23 young foetuses there were only 10 which showed fluorescence. In the group in which no fluorescence was observed the treatment had been given in the patient's home in some cases and in hospital in some. Hence it does not seem probable that the high proportion of cases showing absence of fluorescence is due to carelessness when the medication was given in the patient's home. It may be assumed that there are individual variations in the resorption of tetracycline. The concentration of the drug in the maternal serum varies, and different ratios between the maternal and foetal serum concentrations even seem to occur (Gibbons and Reichelderfer 1960). This assumption is corroborated by the above-mentioned report by Porter *et al.* (1966) according to which fluorescence in the deciduous teeth was not invariably present when protracted tetracycline treatment had been given during the last trimester. It should also be pointed out that although the interval between the medication and the death of the foetus in the present series was usually short, a few weeks at the most, it nonetheless constituted a considerable proportion of the total life of the foetus, and during this period the affected parts of the skeleton may have undergone considerable resorption. Moreover in some cases the treatment was possibly commenced well before the onset of bone mineralization, and hence uptake of the drug might not have taken place. After protracted tetracycline treatment during gestation Johnson and Mitchell (1966) found that the fluorophore disappeared from young rats within three weeks. Considering this and the early time of administration of the tetracycline it is almost surprising that fluorescence was demonstrable in case 170 of the present series. It may be suspected that in this case the tetracycline was ingested later than indicated, or that the patient had received a second course of treatment regarding which no data were available. In the remaining cases showing fluorescence the medication was initiated later and the interval between the termination of medication and the death of the foetus was less than four weeks, i.e. at most about half the time indicated in case 170.

Table II Stillbirths and Neonatal Deaths: Menstrual Age of Tetracycline Administration (2) and of Fetal/Neonatal Death (3) Compound of Tetracycline Used (4) Amount Given per Day (5) and Total Amount (6) Fluorescence Recorded (7) and Some Clinical Comments (8)

Case	Menstrual Age at Med causer	Menstrual Age at Death	Compound of TC used	Amount per Day	Total Amount (g)	Fluorescence Recorded	Clinical Comments
1	2	3	4	5	6	7	8
A	283-284	287	TC	0.25 x 4	2.5		2300 g. Placental insufficiency (?)
B	282-283	287	TC	0.5 x 6	6.0		270 g. Anencephalus
C	185-188	215?	RTC	0.3 x 2	2.4	( )	1120 g. Infarcted placenta

- Gibbons R. J. and Reichelderfer T. E., *Antibiotic Med. Clin. Therapy* 7 618, 1960
- Hughes W. H., Lee W. R. and Flood, M. J. *Brit. J. Pharmacol. Chemotherapy* 25 317 1965
- Solomon R. H. and Mitchell D. F. *Dental Res.* 45 86 1966
- Mich, R. A., Rall D. P. and Tobie J. E. *J. Nat. Cancer Inst.* 19 87 1957
- Porter P. J., Smerny E. A., Golau, H. and Kass E. H. *Antimicrobial agents and Chemotherapy* 1965. Copyright American Society for Microbiology 668, 1966
- Saxén L. *Science* 153, 1384 1966
- Tubero E. *Brit. J. Pharmacol.* 23 445 1964

Received on July 23, 1968

It has been shown that different tetracycline compounds differ in respect to affinity for and effect on the bone tissue (Tubaro 1964 Johnson and Mitchell, 1966 Saxén 1966) In this limited series three different compounds of tetracyclines were used, but the number of commercial brands was much higher *Le* eight. Therefore, and considering the lack of homogeneity in regard to dosage and duration and mode of administration, it is impossible to decide whether any particular compound was more likely than the remainder to leave fluorescence in the human foetal bone tissue. On the other hand, it was apparent that even medication of short duration may leave traces (nos. 147 165 141)

### SUMMARY

A series of 23 fetuses obtained in therapeutic abortion around the menstrual age of 120 days and three perinatally dead infants were examined for tetracycline-type fluorescence in their skeleton. The mothers had received different tetracycline compounds during pregnancy Fluorescence detected in 10 of the cases confirmed earlier findings on the transplacental transmission of the drug, and indicated that this can take place also during the first trimester Individual variations in the time of administration and in the total dose were considerable but there seemed to be a correlation between the dose and the appearance of bone fluorescence Even short term tetracycline administration resulted in fluorescence in some fetuses No differences between the three different tetracyclines used as regards the incorporation in bone could be shown

### REFERENCES

- Berelander G Nakahara H and Rolfe G K. *Develop Biol* 2 289 1960  
de Carneri I Communication to the 2nd Internat Sympos Chemotherapy  
Naples 1961  
Charles D J *Obstet. Gynaec Brit Emp* 61 740 1954  
Cohlan S Q Berelander G and Tamsi T *Amer J Dis Child* 105  
453 1963  
Douglas A C *Brit. J Dis Chest* 57 44 1963  
Frost H M Villanueva A. R and Roth H S *Stam Technol* 35 135  
1960

- Gibbons R. J. and Ratchelderfer T. E. *Antibiotic Med. Clin. Therapy* 7 618, 1960
- Hughes W. H., Lee W. R. and Flood, H. J. *Brit. J. Pharmacol. Chemotherapy* 25 317 1965
- Johnson, R. H. and Mitchell D. F. *Dental Res.* 45 86 1966
- Mitch, R. A., Rall, D. P. and Tobie J. E., *J. Nat. Cancer Inst.* 19 III 1957
- Porter P. J. Sweeney E. A., Golan H. and Kass E. III *Antimicrobial agents and Chemotherapy* 1965 Copyright American Society for Microbiology 668, 1966
- Saxe, L. *Science* 153, 1384 1966
- Tubero E., *Brit. J. Pharmacol.* 23 445 1964

Received on July 25, 1968

## HISTORY OF CONTRACEPTION AND INTRODUCTION OF INTRA UTERINE CONTRACEPTIVE DEVICES IN A RURAL AREA—BANDARAGAMA CEYLON

BY

L. G. ARUMUGAM

Sir Joseph Hutchinson once remarked 'The great remaining challenge of our time is for Man to master the threat of his own numbers'. The modern era of rapid population growth unique both in terms of speed and magnitude in the history of mankind, has its source in the rapid response of the death rate to the modern developments in Science and Technology while the birth rate remains more or less constant. Till quite recently in developing and under-developed countries, survival rather than overgrowth has been the problem. In Ceylon the expectation of life which was about 30 years in 1920 has more than doubled itself due no doubt to the spectacular effectiveness with which modern preventive and curative medicine control disease.

*Population.* A few points regarding the vital statistics in Ceylon may be relevant. Unlike in many other developing countries there is available in Ceylon a century of records relating to vital events.

The enactment of the Births and Deaths Ordinance of 1867 ensured that the occurrence of a birth or death is recorded—a birth within 42 days and a death within 5 days as far as the civil population is concerned. There has been also a decennial census examination commencing in 1871.

The Table appended hereunder shows the rate of population growth from 1871–1967.



Year	Population	Average Growth Rate
1871	2,400,000	1.42
1901	3,658,984	1.72
1931	5,306,871	1.67
1946	6,657,339	1.52
1963	10,644,609	2.75
1967	11,648,148	2.8

After 1946 there is a phenomenal leap upwards and at the present rate of increase, the population will nearly double itself in 25 years. It has even been termed a Demographic Curse for within one year the Island's death rate dropped from 20 per thousand in 1946 to 14.3 in 1947 due in large measure to the dramatic eradication of malaria, the No. 1 killer of that time. Today it is at 8 per thousand, the lowest ever recorded, so that the second half of the present century can be regarded as the epoch of population explosion in Ceylon.

The infant mortality rate declined from 196 per thousand live births in 1900 to 52 in 1967.

The maternal mortality rate of 19 per thousand in 1900 has now fallen to 2.8 in 1964 and is at that level still.

A reference to the population pyramid shows some interesting features. There is a high proportion of young children under 15 years constituting 40 per cent of the total population. The productive group—15–60 years—is about 50 per cent, making the dependency rate high as compared with what obtains in the developed countries.

The number in the infant and pre-school group has more than doubled itself since 1900 so that more and more survive to adolescence and old age.

The birth rate is about 34 per thousand today. A study of fertility trends in Ceylon in 1963–64 revealed that the mean age at marriage was 23.1 in 1960 as compared to 21 in 1900. The total fertility rate was 5.13 in 1952 and 5.07 in 1960. The highest specific fertility rate was among the group aged 25–29 years.

A sudden increase in the rate of growth of the population necessarily entails serious repercussions, politically economically

## HISTORY OF CONTRACEPTION AND INTRODUCTION OF INTRA UTERINE CONTRACEPTIVE DEVICES IN A RURAL AREA—BANDARAGAMA CEYLON

BY

L. G. ARUMUGAM

Sir Joseph Hutchinson once remarked 'The great remaining challenge of our time is for Man to master the threat of his own numbers'. The modern era of rapid population growth unique both in terms of speed and magnitude in the history of mankind, has its source in the rapid response of the death rate to the modern developments in Science and Technology while the birth rate remains more or less constant. Till quite recently in developing and under-developed countries survival rather than overgrowth has been the problem. In Ceylon, the expectation of life which was about 30 years in 1920 has more than doubled itself due no doubt to the spectacular effectiveness with which modern preventive and curative medicine control disease.

*Population:* A few points regarding the vital statistics in Ceylon may be relevant. Unlike in many other developing countries, there is available in Ceylon, a century of records relating to vital events.

The enactment of the Births and Deaths Ordinance of 1867 ensured that the occurrence of a birth or death is recorded—a birth within 42 days and a death within 5 days as far as the civil population is concerned. There has been also a decennial census examination commencing in 1871.

The Table appended hereunder shows the rate of population growth from 1871–1967.

Year	Population	Average Growth Rate
1871	2,400,000	1.42
1901	3,656,954	1.72
1931	5,306,871	1.67
1946	6,657,339	1.52
1963	10,644,809	2.75
1967	11,648,148	2.8

After 1946 there is a phenomenal leap upwards and at the present rate of increase, the population will nearly double itself in 25 years. It has even been termed a Demographic Curse for within one year the Island's death rate dropped from 20 per thousand in 1946 to 14.3 in 1947 due in large measure to the dramatic eradication of malaria, the No. 1 killer of that time. Today it is at 8 per thousand, the lowest ever recorded, so that the second half of the present century can be regarded as the epoch of population explosion in Ceylon.

The infant mortality rate declined from 196 per thousand live births in 1900 to 52 in 1967.

The maternal mortality rate of 19 per thousand in 1900 has now fallen to 2.8 in 1964 and is at that level still.

A reference to the population pyramid shows some interesting features. There is a high proportion of young children under 15 years constituting 40 per cent of the total population. The productive group—15–60 years—is about 50 per cent, making the dependency rate high as compared with what obtains in the developed countries.

The number in the infant and pre-school group has more than doubled itself since 1900 so that more and more survive to adolescence and old age.

The birth rate is about 34 per thousand today. A study of fertility trends in Ceylon in 1963–64 revealed that the mean age at marriage was 23.1 in 1960 as compared to 21 in 1900. The total fertility rate was 5.13 in 1952 and 5.07 in 1960. The highest age specific fertility rate was among the group aged 25–29 years.

A sudden increase in the rate of growth of the population necessarily entails serious repercussions, politically, economically

socially and culturally Ceylon is not as yet self-sufficient in rice—the staple food—and imports are necessary, though in the past few years, food production has been stepped up considerably. Additional factors involved are increasing unemployment, short age of housing and educational problems which the Government has to contend with.

Since the chief factor in the rapid increase of Ceylon's population is fertility, any measures taken to check this would naturally concern the birth rate and family size. There is now a growing awareness of the people to limit the size of their families—this awareness coupled with the governmental effort to disseminate knowledge on planned parenthood, followed by supplies of contraceptives made easily available to the people would produce the desired results.

*History of Family Planning in Ceylon* The Family Planning Association of Ceylon—a voluntary organization—were the pioneers in this work in Ceylon. Their activities commenced in 1953 and the Association has just celebrated its 15th anniversary. In addition to the big, well-attended clinics in Colombo, they also extended their work into other areas, established several clinics and took part in the Training Programme for Doctors.

*Sweden-Ceylon Family Planning Project* This Project commenced its activities in Ceylon after having signed a Bilateral Agreement between the Royal Government of Sweden and the Government of Ceylon in 1958. The terms of the Agreement were for both Governments to co-operate in order to promote and facilitate a pilot project in Community Family Planning in two or more rural areas with a view to extending these activities on a nation wide scale—which has been done since.

Of the areas selected one is a village Bandaragama—about 20 miles from Colombo the capital city. It is a typical agricultural area with a Sinhalese Buddhist population of just over 7000, the literacy rate here being 87 per cent in males and 72 per cent in females.

The second area chosen for its programme of work was the Hindu Indian Tamil populated Tea Estate area of Diyagama, about 100 miles from Colombo. The literacy rate here is much lower.

After a preliminary census and attitude survey a Family Welfare Centre was set up in Bandaragama. The total population in the area at the commencement of activities was 7588 but the Project was concerned only with the Productive Family Group i.e. all couples between the ages of 15 and 40 living as husband and wife and women over 40 who had their last baby within two years and wanted family planning advice. This group is generally 10-12 per cent of the population. The attitude survey revealed that out of 679 families in the Productive Family Group in the village area, 440 or 64.8 per cent were positive to family planning. In 1963-64 75.6 per cent were positive. Families who received more home-visits by the Social Workers remained positive for longer periods.

In regard to the socio-economic background, no difference in attitudes to family planning or fertility was noted.

*Education for the lay population was done in the following manner*

*Wives.* The routine ante-natal talks included facts about family planning, physiology of reproduction and how to avoid pregnancy.

Midwives continued this education to mothers in the homes during their ante-natal and post-partum visits.

Social workers paid regular visits to the home for purposes of motivation.

*Husbands.* Every pregnant woman who attended the ante-natal clinic was given some literature on family planning—enclosed in an envelope and addressed to the husband—to be handed over to him.

Group talks were arranged at suitable intervals.

The co-operation of groups of influential men in the village was sought and they helped with the education of the public.

Films were also shown in order to create an interest in family planning.

*Census.* An annual census was carried out at the end of each year. In order to increase the reliability of the census figures, the project appointed one of its staff members as Census Officer who paid monthly visits to the Registrar of births of the area. The Registrar of marriages was also contacted in order to

socially and culturally Ceylon is not as yet self-sufficient in rice—the staple food—and imports are necessary though in the past few years, food production has been stepped up considerably. Additional factors involved are increasing unemployment, shortage of housing and educational problems which the Government has to contend with.

Since the chief factor in the rapid increase of Ceylon's population is fertility any measures taken to check this would naturally concern the birth rate and family size. There is now a growing awareness of the people to limit the size of their families—this awareness coupled with the governmental effort to disseminate knowledge on planned parenthood, followed by supplies of contraceptives made easily available to the people would produce the desired results.

*History of Family Planning in Ceylon* The Family Planning Association of Ceylon—a voluntary organization—were the pioneers in this work in Ceylon. Their activities commenced in 1953 and the Association has just celebrated its 15th anniversary. In addition to the big, well attended clinics in Colombo they also extended their work into other areas, established several clinics and took part in the Training Programme for Doctors.

*Sweden-Ceylon Family Planning Project* This Project commenced its activities in Ceylon after having signed a Bilateral Agreement between the Royal Government of Sweden and the Government of Ceylon in 1958. The terms of the Agreement were for both Governments to co-operate in order to promote and facilitate a pilot project in Community Family Planning in two or more rural areas with a view to extending these activities on a nation wide scale—which has been done since

Of the areas selected one is a village Bandaragama—about 20 miles from Colombo the capital city. It is a typical agricultural area with a Sinhalese Buddhist population of just over 7000 the literacy rate here being 87 per cent in males and 72 per cent in females.

The second area chosen for its programme of work was the Hindu Indian-Tamil populated Tea Estate area of Diyagama, about 100 miles from Colombo. The literacy rate here is much lower.

Director of Health Services regarding the working and progress of the National Programme.

About two-thirds of the staff have already been trained and 332 clinics have been set up in various parts of the country.

If the programme works to schedule, the target can easily be achieved.

*The Introduction of the Intra-Uterine Contraceptive in Bandaragana.* It was in April 1964 that the I.U.D.-service was introduced in the village area.

Contraception by means of intra-uterine devices is certainly no modern method. For centuries Arabian and Turkish camel-owners have used I.U.D.s to prevent pregnancies in their saddle animals. The technique was simple—a small pebble was inserted into the uterus through a hollow tube.

As early as at the beginning of this century German gynaecologists used this method and in 1924 Grafenberg reported his results with his silver or silk-worm gut rings.

For various reasons, this method became discredited and was not adopted till about 7-8 years ago and since that time no other contraceptive method has undergone so rapid and thorough a change of medical reputation as that experienced by the I.U.D.s. It is unfortunate that Grafenberg never lived to see the acknowledgement of the value of his idea. There is a saying in Medicine that an idea is never accepted because it is good or logical but because the opposition dies off. Two main factors antibiotics and invention of inert plastic materials are mainly responsible for the revival of interest in the Grafenberg ring and its modifications.

In 1959 Oppenheimer of Israel and Ishihama of Japan published reports on I.U.D.s both authors stressed the low pregnancy rates achieved by this method and the absence of any serious side-effects.

In the meantime Margulies of Mt. Sinai Hospital in New York, who had been experimenting for some time with plastic materials, devised a polyethylene spiral, now distributed under the trade name Gynecoil. A little later Dr. Lippes of the University of Buffalo School of Medicine, developed a new polyethylene device in the shape of a double S—known as the Lippes Loop—a

obtain information on newly married couples, with particular references to the trends of migration into and out of the area.

In the village area, the contraceptives supplied in the beginning were condoms foam tablets and diaphragm and jelly. These were issued at the clinic which was incorporated with the Child Welfare Clinic. The methods were taught at the post-natal check up *ie* within 6 weeks of delivery. Other cases motivated by midwives in the field or social workers were also given their supplies here. As the numbers grew it was thought advisable and convenient to do the distribution of foam tablets and condoms in the field by the midwives on their routine home-visits, the idea being to integrate family planning into the existing well-organised Maternal and Child Health services of the country.

In 1964 an evaluation of the work done showed that the birth rate in the village area had dropped from about 30 per thousand in 1958 to 20.4 in 1964. It is reasonable to believe that the declining trend is due to the activities of the Project. The age specific birth rate has also shown a falling trend, particularly for the age group 25-35 years.

Encouraged by these results and the experience gained in the village area, the Swedish Project, in the middle of 1965, suggested to the Government of Ceylon to start Family Planning on a nation-wide scale by integrating it into the Maternal and Child Health services of the country and utilising other health facilities available.

Accordingly a Bilateral Agreement was signed in August 1965 for a period of three years. The aim of the Project's activities are

1. To train medical and other personnel
2. To assist and provide most of the equipment and supplies for the Family Planning clinics
3. To carry out research relating to family planning.

The objective is to reduce the birth rate by a third over a period of 10 years 1966-1976.

The country was divided into 4 regions, each with a population of 2-3.5 million. The Ministry of Health also appointed an Advisory Committee which meets once a month to advise the



Director of Health Services regarding the working and progress of the National Programme.

About two-thirds of the staff have already been trained and 332 clinics have been set up in various parts of the country.

If the programme works to schedule, the target can easily be achieved.

*The Introduction of the Intra-Uterine Contraceptive in Bardagama.* It was in April 1964 that the I.U.D.-service was introduced in the village area.

Contraception by means of intra-uterine devices is certainly no modern method. For centuries Arabian and Turkish camel-owners have used I.U.D.s to prevent pregnancies in their saddle animals. The technique was simple—a small pebble was inserted into the uterus through a hollow tube.

As early as at the beginning of this century German gynaecologists used this method and in 1924 Grafenberg reported his results with his silver or silk-worm gut rings.

For various reasons, this method became discredited and was not adopted till about 7-8 years ago and since that time no other contraceptive method has undergone so rapid and thorough a change of medical reputation as that experienced by the I.U.D.s. It is unfortunate that Grafenberg never lived to see the acknowledgement of the value of his idea. There is a saying in Medicine that an idea is never accepted because it is good or logical but because the opposition dies off. Two main factors antibiotics and in erosion of inert plastic materials are mainly responsible for the revival of interest in the Grafenberg ring and its modifications.

In 1959 Oppenheimer of Israel and Ishihama of Japan published reports on I.U.D.s both authors stressed the low pregnancy rates achieved by this method and the absence of any serious side-effects.

In the meantime Margulies of Mt. Sinai Hospital in New York, who had been experimenting for some time with plastic materials, devised a polyethylene spiral, now distributed under the trade name Gynecoil. A little later Dr. Lippes of the University of Buffalo, School of Medicine developed a new polyethylene device in the shape of a double S—known as the Lippes Loop—a

third type invented by *Birnberg* consists of two triangles joined at the tip and resembling a bow. There is a heart-shaped device, designed by the Pathfinder Fund Association and a host of others.

Unlike the *Grafenberg* silver ring which had to be removed periodically, the new plastic devices are inert and can remain in the uterus indefinitely. They are flexible and can be inserted without dilatation of the cervix, hence no anaesthesia is necessary.

To facilitate removal of the L.U.D. and to determine whether it is in place, *Lippes* attached an appendage of two nylon threads to the loop and *Margulies* had plastic beads on his coil—extending through the cervical canal into the vagina.

Seen in the light of the alarming rate of population growth, what is required is a contraceptive device that is cheap, harmless, reliable, non-repetitive, not related to time of sex union and reversible.

The L.U.D. though not perfect, is nearest this ideal.

*Clinical Observations.* The study at *Bandaragama* includes a group of 2018 women who had their first insertions with *Lippes* Loop C during the period May 1964 to May 1967.

*Propaganda and Motivation.* Ceylon is believed to have the best Maternal and Child Health services in South-East Asia. Trained midwives are posted even in the most remote areas and they are in very close touch with the Productive Family Group—as part of their routine duties, they pay ante-natal and post-natal visits and assist at home deliveries; they also assist at the Child Welfare Clinics and are responsible for immunisations, etc. Thereby, the midwife becomes, more or less, a friend of the family and she it is that has the opportunity to motivate the mother.

All available contraceptive methods are presented to the mother. When the I.U.D. was the method of choice, the woman was referred to the clinic with a note from the midwife. Some of the women, however, were self-referred or sent by their friends, who already had the loop themselves.

The women were advised to attend the clinic soon after or at the tail-end of the menstrual period without sex union as the cervix is partially dilated at that time and there is no risk of

introduction into a pregnant uterus. The device, however, was introduced at any stage of the cycle, provided there was no union.

In cases of lactational amenorrhoea, insertions were done six weeks to two months after delivery.

A preliminary pelvic examination is done to exclude any contra-indications, e.g. suspected pregnancy, history of acute or subacute pelvic inflammatory disease, signs of pelvic inflammation at the time of examination, presence of fibroids, cancer of the cervix clinically evident or suspected, history of recent menorrhagia or metrorrhagia. Cervicitis was not considered a contra-indication to insertion, if there was no sign of infection.

The wearers are taught to examine themselves with washed hands in a squatting position in order to feel the thread before they leave the clinic and are advised to check once in two or three days, especially after the menstrual period, before sex union.

According to age the distribution of cases are

43 per cent under 30 years of age

50 per cent between 30 and 40.

There were no nulliparae that came under our observation. It will be observed that the percentage of younger women accepting the device is now increasing (Table I)

As regards the evaluation of insertions by living children nearly 50 per cent had given birth to three or more living children with an average of 4.5 live births per mother (Tables II, II a, II b)

Table I. Distribution of Cases According to Age

Age group	No of Cases	Per cent
15-19	9	0.5
20-24	319	15.9
25-29	546	27.3
30-34	625	30.9
35-39	416	20.2
40-44	101	5.1
45 & over	2	0.1
	2010	100.0

Bandaragama IUD Acceptors: Per cent by Age by Year 1964-67

Age	May-Dec 1964	Jan-June 1965	July-Dec 1965	Jan-June 1966	July-Dec 1966	Jan-May 1967
19	-	0.3	0.7	0.4	0.9	-
20-24	12.1	13.8	14.8	17.5	17.4	22.0
25-29	24.6	23.9	28.4	26.1	27.5	33.3
30-34	31.3	34.4	32.0	32.5	27.8	22.0
35-39	21.6	23.0	19.7	19.5	21.2	20.6
40+	9.8	4.6	4.5	4.1	5.2	2.1
Total	100.0	100.0	100.0	100.0	100.0	100.0

Note Because of rounding, per cent totals will not always be 100.

Table II Evaluation of Insertion by Living Children

Living Children	No. of Cases	Per cent
1	126	6.2
2	297	14.7
3	384	19.3
4	406	20.1
5	315	15.6
6	219	10.8
7	135	6.6
8	1	3.5
9+	65	3.2
Total	2018	100.0

Table II a. Evaluation of Insertion by Parity

Parity	No of Cases	Percentage
1	102	5.0
2	251	12.4
3	325	16.2
4	34	1.7
5	354	17.5
6	22	1.1
7	175	8.7
8	94	4.7
9+	145	7.2
Total	2018	100.0

Table II b. L.U.D. Insertions by Age/Parity

Parity Age	0	1	2	3	4	5	6	7	8	9	Total
15-19		4	5			-	-	-		-	9
20-24	53	112	92	42	14	4			-	-	319
25-29	24	87	133	120	99	43	31	5	4		546
30-34	14	40	81	134	149	98	58	23	28		625
35-39	5	7	21	37	60	69	71	54	72		416
40-44			1	9	12	13	15	10	41		101
45						-	-		2		2
Total	102	251	328	342	354	227	175	94	145		2018

Nearly two thirds of the women wish to limit their families rather than space (Table III). A summary of trends 1964-1967 shows:

- younger women are accepting the L.U.D.
- a greater proportion accepting the L.U.D. for limiting of family size.
- family size preferences are decreasing.

The data obtained during recent years of reports published concerning the modern L.U.D. shows that the method has its limitations.

What are the side-effects or complications?

Expulsions (Table IV) include complete expulsions into or from the vagina or partial expulsions requiring removal of the I.U.D. from the cervix, whether noticed or unnoticed by the wearer. In our observations, out of a total of 303 first expulsions, 200 or nearly two thirds had the device re-inserted. In the case of re-insertions Loop D which is slightly larger than C was used.

Of 47 second expulsions, 30 had re-insertions and of 14 third expulsions 9 had re-insertions.

The majority of first expulsions took place in the period 0-5 months, but it will be observed that there were almost an equal number which occurred in the period 6-12 months after insertion.

Bandaragama I.U.D. Acceptors, Per cent by Age by Year 1964-67

Age	May-Dec 1964	Jan-June 1965	July-Dec 1965	Jan-June 1966	July-Dec 1966	Jan-May 1967
19	-	0.3	0.7	0.4	0.9	-
20-24	12.1	13.8	14.8	17.5	17.4	22.0
25-29	24.6	23.9	28.4	26.1	27.5	33.3
30-34	31.8	34.4	32.0	32.5	27.8	22.0
35-39	21.6	23.0	19.7	19.5	21.2	20.6
40+	9.8	4.6	4.5	4.1	5.2	2.1
Total	100.0	100.0	100.0	100.0	100.0	100.0

Note Because of rounding, per cent totals will not always be 100

Table II. Evaluation of Insertion by Living Children

Living Children	No. of Cases	Per cent
1	126	6.2
2	297	14.7
3	384	19.3
4	406	20.1
5	315	15.6
6	219	10.8
7	135	6.6
8	71	3.5
9+	65	3.2
Total	2018	100.0

Table IIa. Evaluation of Insertion by Parity

Parity	No of Cases	Percentage
1	102	5.0
2	251	12.4
3	328	16.2
4	342	17.1
5	354	17.5
6	227	11.2
7	175	8.7
8	94	4.7
9+	145	7.2
Total	2018	100.0

Removals have been done in 126 cases (Table IV) for the following reasons.

*Bleeding.* The most important medical reason for removal is bleeding, including spotting over long periods. Most of the side-effects not requiring removal also come under this category as will be seen in Table V. Of a total of 126 removals for various reasons, 33 per cent come under this heading.

Table IV Removals by Reason and Months of Use

Reasons	Months of Use														Total
	0-2	3-5	6-8	9-11	12	14	15-17	18-20	21	23	24-26	27	29	30+	
Bleeding	10	9	8	6	2	2		4		2	1	3		47	
Abdominal Pain	2		1							-	-			3	
Wants Baby			2	2	6	4		5	2	-	4	1		26	
Leucorrhoea	1				1	1								3	
Husband's Request	3	4		1	1	2			2					13	
Personal	1	4		2	2			1				2		12	
Medical		1	2					2	1	-	1	1		8	
Frequent		1	4	1	1			1						8	
Irregular Periods			3										1	4	
Others	1		1				1					-	-	2	
Infusion				N			1			L					
Total	18	22	18	12	13	9	13	5	2	6	8			126	

Table V Side Effects not Requiring Removal

Spotting	125
Rach Periods	111
Bleeding	9
Irregular Periods	36
Leucorrhoea	11
Back Ache	11
Abdominal Pain	12
Others	13
Total	328

Table III. *Bandaragama, IUD Acceptors by Intent to Space or Limit Child Bearing by Year 1964-67*

Year	Space	Limit	Total
1964 (May-Dec)	52 (19.7%)	211	263
1965	177 (23.5%)	575	52
1966	254 (29.7%)	599	553
1967 (Jan-May)	47 (33.3%)	94	141
	530 (26.3%)	1479	2009

Information incomplete for 9 acceptors

Year	Limiters Number of Living Children			
	1-2	3-4	5+	Total
1964 (May-Dec)	4.7	32.7	52.5	100 (N=211)
1965	4.7	42.2	53.0	100 (N=575)
1966	6.3	42.7	50.9	100 (N=599)
1967 (Jan-May)	10.6	48.9	40.4	100 (N=94)

Table III a. *Expulsions by Months of Use and Re-insertions*

	1st Expul- sion	Re-Inser- tion	2nd Expul- sion	Re-Inser- tion	3rd Expul- sion	Re-Inser- tion
0-2	78	51	18	16	5	4
3-5	68	46	12	4	2	7
6-8	41	30	8	5	2	2
9-11	33	22	3	3	-	-
12-14	43	27	4	1	4	1
15-17	19	10	2	2	-	-
18-20	14	9	-	-	1	-
21-23	7	4	-	-	-	-
24+	6	-	-	-	-	-
Total	303	199	47	31	14	9

Investigation showed that most of the expulsions occurred around the time of the menses and were complete rather than partial.

Tietze, in his analyses showed an inverse relationship between parity and age to expulsion rates. The age of the woman, however appears to be a stronger factor



Removals have been done in 126 cases (Table IV) for the following reasons

**Bleeding.** The most important medical reason for removal is bleeding, including spotting over long periods. Most of the side-effects not requiring removal also come under this category as will be seen in Table V. Of a total of 126 removals for various reasons, 33 per cent come under this heading.

Table IV Removals by Reason and Months of Use

Reasons	Months of Use													Total
	0-2	3-5	6-8	9-11	12	14	15-17	18-20	21-23	24-26	27	29	30+	
Bleeding	10	9	8	6	2	2		4		2	1	3		47
Abdominal														
Pain	2		1							-		-		3
Wants Baby			2	2	6	4		5	2		4	1		26
Leucorrhoea	1				1	1								3
Husband's														
Request	3	4		1	1	2			2			-		13
Personal	1	4		2	2			1				2		12
Medical		1	2					2	1		1	1		8
Pregnant		1	4	1	1			1						8
Irregular														
Periods			3											
Others	1		1										1	4
Infection					N		1		L					2
Total	18	22	10	12	13		9	13	5	2	6	8		126

Table V Side Effects not Requiring Removal

Spotting	125
Rach Periods	111
Bleeding	9
Irregular Periods	36
Leucorrhoea	11
Back Ache	11
Abdominal Pain	12
Others	13
Total	328

Table III. Bandaragama. IUD Acceptors by Intent to Space or Limit Child Bearing by Year 1964-67

Year	Space	Limit	Total
1964 (May-Dec)	52 (19.7 %)	211	263
1965	177 (23.5 %)	575	752
1966	254 (29.7 %)	599	853
1967 (Jan-May)	47 (33.3 %)	94	141
	530 (26.3 %)	1479	2009

Information incomplete for 9 acceptors

Year	Limiters Number of Living Children				
	1-2	3-4	5+	Total	
1964 (May-Dec)	47	32.7	62.5	100	(N=211)
1965	47	42.2	53.0	100	(N=575)
1966	6.3	42.7	50.9	100	(N=599)
1967 (Jan-May)	10.6	48.9	40.4	100	(N=94)

Table III a. Expulsions by Months of Use and Re-Inscriptions

	1st Expulsion	Re-Inscription	2nd Expulsion	Re-Inscription	3rd Expulsion	Re-Inscription
0-2	78	51	18	16	5	4
3-5	68	46	12	4	2	2
6-8	41	30	8	5	2	2
9-11	33	22	3	3	-	-
12-14	43	27	4	1	4	1
15-17	18	10	2	2	-	-
18-20	14	9	-	-	1	-
21-23	7	4	-	-	-	-
24+	8	-	-	-	-	-
Total	308	199	47	31	14	9

Investigation showed that most of the expulsions occurred around the time of the menses and were complete rather than partial.

Tietze in his analyses showed an inverse relationship between parity and age to expulsion rates. The age of the woman however appears to be a stronger factor.

section. The bleeding usually stops after the second menstrual period after insertion. This was more marked in anaemic wearers. Calcium and Iron Tablets were given and mostly reassurance to tide the patient over the first three months of use. The bleeding is not usually of an alarming quantity but sufficient to create fear and anxiety in the patient.

*Irregular periods.* 36 had irregular periods for some months following insertion but they did not want the device removed on that score.

The other side-effects were back-ache, leucorrhoea and abdominal pain.

*Follow-Up* Wearers are advised to return for follow-up one month after insertion, the main reason being to see how they have reacted to the device, i.e. whether they have had any side-effects or not.

The second visit is three months after the first, because most of the side effects, if any have subsided by that time. They are advised to report once in six months or so in order to calculate the woman months of use.

Prior to this present evaluation of our work, since the follow-up was considered incomplete, a special follow-up survey was organised. The statistician of the Project devised a special Form and a house-to-house survey was conducted by trained workers under her supervision.

By this means we were able to get accurate information about the cases. Many of the cases who came from outside our area were contacted by mail and surprisingly enough, many of them replied. It might be mentioned that a large number of this group were midwives and nurses who had got the insertion done at Bandaragama during their training period.

Many of the wearers who did not turn up for their follow-ups were those who had no complaints at all and did not bother—a few however were those who had expelled the device and took to another method or gave up family planning methods altogether.

Sixty two in this whole group, i.e. 6 per cent, were lost to all follow-up.

*Closed cases (Table V1)* These rates are based on all events—expulsions, pregnancies and removals.

*Abdominal Pain.* Only three women had the device removed for abdominal pain. In passing, I might mention that we had very little experience of abdominal pain or cramps after insertion.

*Planning pregnancy* 27 were planning pregnancies—mainly after the lapse of a year or more except in a few cases where the age of the last child was over two years.

*Husband's objections* Mainly in those cases where the wife had the device inserted without the husband's prior knowledge.

*Medical Reasons* for removal other than bleeding include pre-existing and intercurrent conditions requiring treatment clearly unrelated to the IUD as well as vague complaints related to the IUD by the patient.

*Accidental Pregnancies.* In this group are included those accidental pregnancies that occur with the loop *in situ* or with the device undetermined *i.e.* after an unnoticed expulsion. Of the 27 pregnancies that occurred in the group under observation, 20 were with the loop *in situ*.

Of these 8 had the loop removed on request this was done easily 14 had normal deliveries.

2 ended in abortions (these were not the ones that had the loop removed)

1 left the area and was lost to follow-up

1 miscarriage—5th month.

2 had not delivered at the end of 1967

Of the 7 with the device undetermined 6 had normal deliveries.

1 had not delivered at the end of 1967

*Ectopic pregnancies.* None were observed in this group

*Perforations.* None were recorded in this series.

*Infection* There were no cases of pelvic infection observed in this series

*Side-Effects* 16 per cent of the cases under observations had side-effects, which did not warrant removal (Table V)

*Spotting* 125 cases or about 6 per cent had spotting of varying duration *i.e.* from a few days to a month or even longer

*Rich Periods.* Bleeding is a bit of a problem 111 had rich periods which occur generally for the first two months after an

In the first year the first expulsion rate is 15.8 (rather high) but closure rate is only 6.2.

Total closure rates are 11.3 at the end of 12 months and 19.8 at the end of 24 months, so that the corresponding continuation rates are 88.7 and 80.2.

Table 7 shows a comparison of net cumulative continuation rates per 100 acceptors in Bandaragama and other various programmes, *i.e.* in Co-operative Statistical programme for evaluation of Intra-Uterine Devices.

Our continuation rates are good—88.7 at the end of 12 months and 80.2 at the end of 24 months.

### SUMMARY

The chief advantage of the I.U.D. as a contraceptive method is that once the insertion is done and as long as the I.U.D. remains in place no further contraceptive action is required.

Other points in its favour are

- a. high level of protection
- b. easy insertion and removal
- c. low cost of production
- d. complete stability under all climatic conditions.

The I.U.D. has been endorsed as a method of contraception by national and international health authorities.

The I.U.D. is particularly advantageous to a population that is not accustomed to contraceptive practice is economically disadvantaged, where literacy rate is low and medical services in short supply.

### *Acknowledgements*

Opportunity is taken to thank Dr C. E. Groth, Director and Mr A. E. Q. Helling, Administrative Secretary of the Sweden-Ceylon Family Planning Project, for their kind assistance in enabling special surveys to be carried out which has contributed to make the data submitted in this Report complete.

Dr Nicholas Wright, Resident Representative of the Population Council, must also be thanked for helping in the analysis of the figures.

Table VI. *Net Cumulative Event and Closure Rates by Type of Termination Continuation Rates and Cumulated Months of Use by Duration of Use—Bandaragama Ceylon*

Type of Termination	Rate per 100 Women	
	12th Month	24th Month
<i>Events</i>		
Pregnancy	0.6	1.8
Expulsions:		
First	15.8	23.1
Later	2.0	5.4
Removals		
Medical	3.1	4.6
Planning Pregnancy	0.2	1.3
Other Personal	1.4	2.7
Months of Use Cumulated	6810	13,107
<i>Closures</i>		
Pregnancy	0.6	1.6
Expulsions:		
First	6.2	9.7
Later	0.5	2.2
Removals		
Medical	2.5	3.0
Planning Pregnancy	0.2	1.3
Other Personal	1.3	2.0
Total Closures	11.3	19.8
Continuation Rate	88.7	80.2

Table VII. *Net Cumulative Continuation Rates per 100 Acceptors at 1, 2 and 3 Years in Various Programmes*

	1 year	2 years	3 years
Bandaragama	89.9	80.2	-
CSP (U.S.)	78.5	66.6	58.5
Taiwan	68.8	57.7	-
Thailand	78.0	59.6	-
Pakistan	78.6	61.2	-

Co-operative Statistical Programme for the Evaluation of Intra-Uterine Devices

Source: Retention of IUDs: An International Comparison. Studies in Family Planning, Number 18 April 1967

In the first year the first expulsion rate is 15.8 (rather high) but closure rate is only 6.2.

Total closure rates are 11.3 at the end of 12 months and 19.8 at the end of 24 months, so that the corresponding continuation rates are 88.7 and 80.2.

Table 7 shows a comparison of net cumulative continuation rates per 100 acceptors in Bandaragama and other various programmes, *Le* in Co-operative Statistical programme for evaluation of Intra-Uterine Devices.

Our continuation rates are good—88.7 at the end of 12 months and 80.2 at the end of 24 months.

### SUMMARY

The chief advantage of the LUD as a contraceptive method is that once the insertion is done and as long as the LUD remains in place, no further contraceptive action is required.

Other points in its favour are

- a. high level of protection
- b. easy insertion and removal
- c. low cost of production
- d. complete stability under all climatic conditions.

The LUD has been endorsed as a method of contraception by national and international health authorities.

The LUD is particularly advantageous to a population that is not accustomed to contraceptive practice, is economically disadvantaged, where literacy rate is low and medical services in short supply.

### Acknowledgements

Opportunity is taken to thank Dr C. E. Groth, Director and Mr A. E. Q. Helling, Administrative Secretary of the Sweden-Ceylon Family Planning Project, for their kind assistance in enabling special surveys to be carried out which has contributed to make the data submitted in this Report complete.

Dr Nicholas Wright, Resident Representative of the Population Council, must also be thanked for helping in the analysis of the figures.

Table VI. Net Cumulative Events and Closure Rates by Type of Termination Continuation Rates and Cumulated Months of Use by Duration of Use—Bandaragama Ceylon

Type of Termination	Rate per 100 Women	
	12th Month	24th Month
<b>Events</b>		
Pregnancy	0.6	1.8
Expulsions		
First	15.8	23.1
Later	2.0	5.4
Removals		
Medical	3.1	4.6
Planning Pregnancy	0.2	1.3
Other Personal	1.4	2.7
Months of Use Cumulated	6810	13,107
<b>Closures</b>		
Pregnancy	0.6	1.6
Expulsions		
First	6.2	9.7
Later	0.5	2.2
Removals		
Medical	2.5	3.0
Planning Pregnancy	0.2	1.3
Other Personal	1.3	2.0
Total Closures	11.3	19.8
Continuation Rate	68.7	80.2

Table VII. Net Cumulative Continuation Rates per 100 Acceptors at 1, 2 and 3 Years in Various Programmes

	1 year	2 years	3 years
Bandaragama	89.9	80.2	
CSP (U.S.)	78.3	66.6	58.5
Taiwan	68.8	57.7	
Thailand	78.0	59.6	
Pakistan	78.6	61.2	

Co-operative Statistical Programme for the Evaluation of Intra-Uterine Devices.

Source: "Retention of IUDs: An International Comparison Studies in Family Planning, Number 18 April 1967"



In the first year the first expulsion rate is 15.8 (rather high) but closure rate is only 6.2.

Total closure rates are 11.3 at the end of 12 months and 19.8 at the end of 24 months, so that the corresponding continuation rates are 88.7 and 80.2.

Table 7 shows a comparison of net cumulative continuation rates per 100 acceptors in Bandaragama and other various programmes, i.e. in Co-operative Statistical programme for evaluation of Intra-Uterine Devices.

Our continuation rates are good—88.7 at the end of 12 months and 80.2 at the end of 24 months.

### SUMMARY

The chief advantage of the L.U.D. as a contraceptive method is that once the insertion is done and as long as the L.U.D. remains in place, no further contraceptive action is required.

Other points in its favour are

- a. high level of protection
- b. easy insertion and removal
- c. low cost of production
- d. complete stability under all climatic conditions.

The L.U.D. has been endorsed as a method of contraception by national and international health authorities.

The L.U.D. is particularly advantageous to a population that is not accustomed to contraceptive practice, is economically disadvantaged, where literacy rate is low and medical services in short supply.

### Acknowledgements

Opportunity is taken to thank Dr C. E. Groth, Director and Mr A. E. Q. Helling, Administrative Secretary of the Sweden-Ceylon Family Planning Project, for their kind assistance in enabling special surveys to be carried out which has contributed to make the data submitted in this Report complete.

Dr Nicholas Wright, Resident Representative of the Population Council, must also be thanked for helping in the analysis of the figures.

Table VI. *Net Cumulative Event and Closure Rates by Type of Termination Continuation Rates and Cumulated Months of Use by Duration of Use-Bandaragama Ceylon*

Type of Termination	Rate per 100 Women	
	12th Month	24th Month
<i>Events</i>		
Pregnancy	0.8	1.3
Expulsions:		
First	15.8	23.1
Later	2.0	5.4
Removals		
Medical	3.1	4.6
Planning Pregnancy	0.2	1.3
Other Personal	1.4	2.7
Months of Use Cumulated	8810	13,107
<i>Closures</i>		
Pregnancy	0.6	1.6
Expulsions		
First	6.2	9.7
Later	0.5	2.2
Removals		
Medical	2.5	3.0
Planning Pregnancy	0.2	1.3
Other Personal	1.3	2.0
Total Closures	11.3	19.8
Continuation Rate	88.7	80.2

Table VII. *Net Cumulative Continuation Rates per 100 Acceptors at 1, 2 and 3 Years in Various Programmes*

	1 year	2 years	3 years
Bandaragama	88.9	80.2	-
CSP (U.S.)	78.5	66.6	58.5
Taiwan	68.8	57.7	-
Thailand	78.0	59.6	-
Pakistan	78.6	61.2	-

Co-operative Statistical Programme for the Evaluation of Intra-Uterine Devices

Source: "Retention of I.U.D.s: An International Comparison Studies in Family Planning, Number 18 April 1967"

In the first year the first expulsion rate is 15.8 (rather high) but closure rate is only 6.2.

Total closure rates are 11.3 at the end of 12 months and 19.8 at the end of 24 months, so that the corresponding continuation rates are 88.7 and 80.2.

Table 7 shows a comparison of net cumulative continuation rates per 100 acceptors in Bandaragama and other various programmes i.e. in Co-operative Statistical programme for evaluation of Intra-Uterine Devices.

Our continuation rates are good—88.7 at the end of 12 months and 80.2 at the end of 24 months.

### SUMMARY

The chief advantage of the I.U.D. as a contraceptive method is that once the insertion is done and as long as the I.U.D. remains in place, no further contraceptive action is required.

Other points in its favour are

- a. high level of protection
- b. easy insertion and removal
- c. low cost of production
- d. complete stability under all climatic conditions.

The I.U.D. has been endorsed as a method of contraception by national and international health authorities.

The I.U.D. is particularly advantageous to a population that is not accustomed to contraceptive practice, is economically disadvantaged, where literacy rate is low and medical services in short supply.

### Acknowledgements

Opportunity is taken to thank Dr C. E. Groth, Director and Mr A. E. Q. Helling, Administrative Secretary of the Sweden-Ceylon Family Planning Project, for their kind assistance in carrying out special surveys to be carried out which has contributed to make the data submitted in this Report complete.

Dr Nicholas Wright, Resident Representative of the Population Council, must also be thanked for helping in the analysis of the figures.

Table VI. *Net Cumulative Event and Closure Rates by Type of Termination Continuation Rates and Cumulated Months of Use by Duration of Use—Bandaragama Ceylon*

Type of Termination	Rate per 100 Women	
	12th Month	24th Month
<i>Events</i>		
Pregnancy	0.6	1.8
Expulsions:		
First	15.8	23.1
Later	2.0	5.4
Removals		
Medical	3.1	4.6
Planning Pregnancy	0.2	1.3
Other Personal	1.4	2.7
Months of Use, Cumulated	6810	13 107
<i>Closures</i>		
Pregnancy	0.6	1.6
Expulsions:		
First	6.2	9.7
Later	0.5	2.2
Removals		
Medical	2.5	3.0
Planning Pregnancy	0.2	1.3
Other Personal	1.3	2.0
Total Closures	11.3	19.8
Continuation Rate	68.7	60.2

Table VII. *Net Cumulative Continuation Rates per 100 Acceptors at 1, 2 and 3 Years in Various Programmes*

	1 year	2 years	3 years
Bandaragama	89.9	80.2	—
CSP (U.S.)	78.5	66.6	58.5
Taiwan	63.8	57.7	—
Thailand	78.0	59.6	—
Pakistan	78.6	61.2	—

Co-operative Statistical Programme for the Evaluation of Intra Uterine Devices

Source: Retention of IUDs: An International Comparison. Studies in Family Planning, Number 18. April 1967.

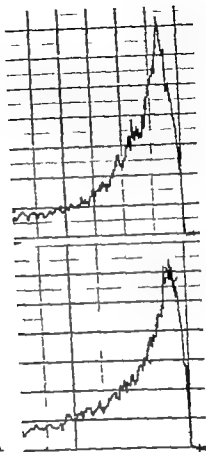


Fig 1 Bilaterally normal renogram.

33 ovarian tumour in 15 and inflammatory adnexal disease in 13. The interval from hysterectomy to the follow-up examination varied from a fortnight to 18 months. In 80 per cent, a minimum of 4 months had elapsed from the operation. Sixty-five of the patients seen at the follow-up had been operated on by a qualified gynaecologist and 215 by a house surgeon, specializing in gynaecology. The abdominal hysterectomies by the qualified surgeons were mostly carried out using the extrafascial method whereas the others were almost without exception performed by the intrafascial method.

## REFERENCES

- Kinch Anne* Report, Sweden-Ceylon Family Planning Project 1966  
*Tietze Christopher* Contraception with Intra-Uterine Devices  
*Tornberg, Georg*, Preliminary Report on Sweden-Ceylon Family Planning Project 1964  
- Sociological Report 1965  
*Wright N* History of Contraceptive Practice among Post Partum Loop Acceptors Population Council-L.U.D. Recommended Procedures for Data Analysis-April 1967  
Taiwan Population Study Centre F.P. in Taiwan 1965-66

Received on June 21 1968

## RENOGRAPHIC STUDIES OF URETERIC PATENCY FOLLOWING TOTAL HYSTERECTOMY

BY

OSMO KOSKELA, JUHA KOKKONEN AND JAAKKO VAHALA

It is known that radical hysterectomy for cancer of the uterine cervix exposes the ureters to a risk of injury. Ureteric lesions are sometimes seen in the form of fistulae even after hysterectomy for benign gynaecologic condition. They pose the gynaecologist a considerable therapeutic, but seldom any diagnostic problem. The part played by hysterectomy in the aetiology of operative ureteric lesions is aptly illustrated by Higgins material (1967) with its 87 postoperative ureteric lesions of which 50 followed hysterectomy.

Follow-up examinations after hysterectomy for mainly benign conditions (Morrisson 1960, Solomons *et al.* 1960, Dahls *et al.* 1962) have revealed transient and sometimes permanent, ureteric lesions which may have been completely symptom-free or given such mild symptoms as would have been overlooked without a routine follow-up examination of the ureters postoperatively. The earlier methods of examination were intravenous pyelography (Morrisson 1960, Solomons *et al.* 1960, Dahls *et al.* 1962) or cystoscopy with intravenous pyelography only in selected cases (St Martin *et al.* 1953, Freda and Tacchi 1962).

Gerble and Flanagan (1962) and Brattenberg *et al.* (1965) used radiolotope renography to examine postoperative ureteric conditions. The indicator they used was Hippuran<sup>®</sup> labelled with I-131.

The reliability of radiolotope renography for examination of ureteric capacity to convey urine was demonstrated, by com-

paring it with the pyelographic findings, e.g. by Denneberg (1960), Zum Winkel and Scheer (1960) and Lawrence *et al.* (1963). It is mainly the declining part of the renographic curve which is considered to indicate the ureteric capacity for conveying urine. The curve may decline very slowly suggesting an incomplete obstacle to urinary flow. If the curve goes on rising throughout the examination, there is a complete obstruction to urinary flow while demonstrable renal function still exists (Winter, 1963; Zum Winkel 1964).

Isotope renography is believed to reveal any disorders of ureteric flow even earlier than intravenous pyelography and this method is considered highly suitable for observation of the emptying of the upper urinary tract (Zum Winkel *et al.* 1960; Denneberg, 1960; Feine and Bauer 1961; Gerble and Flanagan 1962; Braitenberg *et al.* 1965).

The purpose of the present study was to illustrate with the aid of isotope renography the incidence of latent and permanent ureteric lesions possibly produced by total hysterectomy in treatment of benign conditions.

### Material

During the period Dec. 1 1965 to Aug. 31 1967 total hysterectomy for benign diseases was carried out at the Department of Obstetrics and Gynaecology of Oulu Provincial Hospital on 300 patients either vaginally or by laparotomy. All the 300 consecutive patients of whom 222 had been operated on by the abdominal and 78 by the vaginal route were invited to undergo follow-up examination in order to verify the condition of their ureters by isotope renography. 280 patients (93.3 per cent) attended the follow-up examination. In 76 hysterectomy had been performed by the vaginal route. Vaginal hysterectomy had always been accompanied by vaginoplasty. Of the patients treated by laparotomy 56 had undergone hysterectomy only, 35 hysterectomy and unilateral salpingo-oophorectomy, while in 113 cases both adnexae had been removed at the time of hysterectomy.

The principal diagnoses at laparotomy were uterine fibroids in 112 cases, recurrent irregular bleeding in 31, endometriosis in



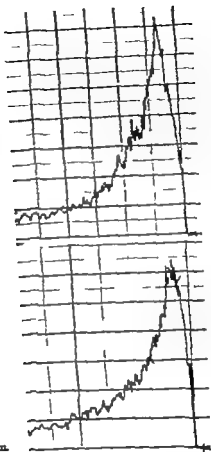


Fig 1 Bilaterally normal renogram.

33 ovarian tumour in 15 and inflammatory adnexal disease in 13. The interval from hysterectomy to the follow-up examination varied from a fortnight to 18 months. In 80 per cent a minimum of 4 months had elapsed from the operation. Sixty-five of the patients seen at the follow-up had been operated on by a qualified gynecologist and 215 by a house surgeon, specializing in gynaecology. The abdominal hysterectomies by the qualified gynecologists were mostly carried out using the extrafascial method, whereas the others were almost without exception performed by the intrafascial method.

paring it with the pyelographic findings e.g. by Denneberg (1960), Zum Winkel and Scheer (1960) and Lawrence *et al* (1963). It is mainly the declining part of the renographic curve which is considered to indicate the ureteric capacity for conveying urine. The curve may decline very slowly suggesting an incomplete obstacle to urinary flow. If the curve goes on rising throughout the examination, there is a complete obstruction to urinary flow while demonstrable renal function still exists (Winter 1963, Zum Winkel 1964).

Isotope renography is believed to reveal any disorders of ureteric flow even earlier than intravenous pyelography and this method is considered highly suitable for observation of the emptying of the upper urinary tract (Zum Winkel *et al* 1960, Denneberg 1960, Feine and Bauer 1961, Gerbie and Flanagan 1962, Braitenberg *et al* 1965).

The purpose of the present study was to illustrate with the aid of isotope renography the incidence of latent and permanent ureteric lesions possibly produced by total hysterectomy in treatment of benign conditions.

### *Material*

During the period Dec. 1 1965 to Aug. 31 1967 total hysterectomy for benign diseases was carried out at the Department of Obstetrics and Gynaecology of Oulu Provincial Hospital on 300 patients either vaginally or by laparotomy. All the 300 consecutive patients of whom 222 had been operated on by the abdominal and 78 by the vaginal route were invited to undergo follow-up examination in order to verify the condition of their ureters by isotope renography. 280 patients (93.3 per cent) attended the follow-up examination. In 76 hysterectomy had been performed by the vaginal route. Vaginal hysterectomy had always been accompanied by vaginoplasty. Of the patients treated by laparotomy 56 had undergone hysterectomy only, 35 hysterectomy and unilateral salpingo-oophorectomy, while in 113 cases both adnexae had been removed at the time of hysterectomy.

The principal diagnoses at laparotomy were uterine fibroids in 112 cases, recurrent irregular bleeding in 31, endometriosis in

determined beforehand by X-ray examination. The collimator tubes were placed against the probable sites of the kidneys, and their position was changed in the early phases of the examination if the site of maximum activity differed from their initially assumed positions. Abnormal renograms were repeated, and if the curve was still pathological on either side the condition of the ureters was verified by intravenous pyelography (Figs. 1 and 2)

### Results

The results are presented in Table I. The renograms of 241 patients were normally shaped. For another 26 the repeated renograms were normal. In all these cases the half time of activity bilaterally was less than 18 minutes. The remaining 13 patients underwent intravenous pyelography with special attention focused on the condition of the ureters along their whole course to the bladder. The ureters were normal in the pelvic region of all but one of these 13. In this case the right ureter deviated medially but was patent and smooth-walled. A unilateral mobile kidney was detected in two patients, a large calculus in the renal pelvis in one while renal changes caused by chronic pyelonephritis were noted in two patients. One patient had ureteric narrowing at the junction between the renal pelvis and the ureter which explained the repeatedly abnormal unilateral renogram (Fig. 2). Two patients had high blood pressure and one compensated cardiac failure. In five patients whose renograms repeatedly showed a pathological shape, mainly an abnormal ascending part, intravenous pyelography offered no explanation for the changes in the renogram. Group I of Table I included a patient in whom a ureterovaginal fistula caused by total hysterectomy had been

Table I The Total Hysterectomy Series Examined Postoperatively by Renography and Intravenous Pyelography

First postoperative renogram bilaterally normal	241
Follow-up renograms bilaterally normal	26
Ureters condition verified by intravenous pyelography and ureters in the pelvic region found normal	13
Total attending follow-up examination	280

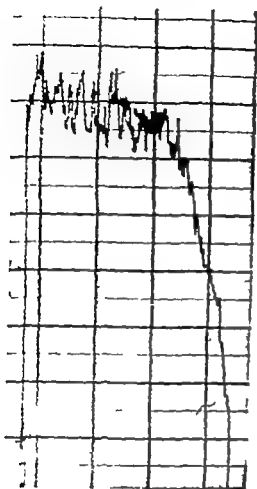


Fig. 2. Renogram of a patient in whom ureteric narrowing was found at the borderline between renal pelvis and ureter

### Method

The patients seen for follow up examination were subjected to radioisotope renography using Hippuran<sup>®</sup> (sodium-ortho-iodohippurate) labelled with  $I^{131}$  0.5  $\mu$ Ci per kg of body weight was injected into the cubital vein. The examination was carried out in the supine position, and the variation of activity in both renal regions was observed separately by external measurements for 15–30 minutes. The activity recorder used was Berthold's two-channel renograph. Its scintillation detectors were equipped with sodium iodide crystals activated by thallium. The collimator openings were 5 cm in diameter. The site of the kidneys was not

Table II. Incidence of Ureteric Damage in three Consecutive Series of Total Hysterectomies

Author	Number of Operations	Permanent Latent Lesions	Transient Lesions	Fistulas
Solomons <i>et al.</i>	191	3	2	
Dahle <i>et al.</i>	98	1	not reliably examined	2 (one vesico-vaginal)
Kocherle <i>et al.</i>	280		not reliably examined	1

Abdominal total hysterectomy is considered to constitute a graver risk to the ureters than the vaginal operation, mainly due to the different indications for these two operations. The absence of latent ureteric lesions in the present series may be attributed partly to the fact that in our hospital the less experienced surgeons always carried out abdominal hysterectomy for benign diseases by the intrafascial method.

Isotope renography is a simple, quick and reliable examination involving no discomfort to the patient. Without any preparations, it may be used to verify the condition of the ureters postoperatively. Patients in poor condition, and even patients sensitive to contrast media who therefore cannot undergo intravenous pyelography can be examined by this method. The amount of irradiation involved in isotope renography by Hippuran is considered negligible. Qualitative assessment is considered reliable in the interpretation of the curves (Gerbitz and Flanagan 1962 Winter 1963 Koplowitz *et al.* 1965). Besides qualitative assessment, the half-times of the renograms were also measured in the present follow-up examination: those not exceeding 18 minutes, according to Zum Winkel (1964) were considered normal.

Although no latent, permanent ureteric lesions were detected in the present series the literature seems to justify the statement by Fedt and Tacchi "Whether or not an incidence of unrecognized non-functioning ureters of almost 2 per cent is suf

repaired by a successful operation prior to the follow-up examination. The fistula had originated in connection with extrafascial hysterectomy performed by laparotomy.

### Discussion

In symptom-free operative ureteric lesions, diagnosis can only be reached by routine examination of the postoperative condition of the ureters. Reliable statistics, analysing the effect of hysterectomy on the ureters, must be based on material including a preoperative examination. Hysterectomies for malignant growths are not accepted for inclusion in series of this type (Solomons *et al.* 1960, Dahle *et al.* 1962).

Only 6.7 per cent of the patients invited failed to attend the postoperative follow-up examination of the ureters. The present material may therefore reliably be considered to represent the effect on the ureters of 300 consecutive total hysterectomies for benign diseases. The follow-up examination revealed not one latent ureteric lesion. The lack of preoperative examination of the ureters of the present patients is therefore of no importance.

It is difficult to find in the literature any comparable statistics on ureteric lesions caused by total hysterectomy although routine postoperative examination has been carried out in many series. The series reported on by *Sr Martin et al.* (1953), *Morrison* (1960) and *Freda and Tacchi* (1962) contained cases of malignancy. Both these series and that reported on by *Solomons et al.* (1960) also contained other gynaecological operations. The routine examinations on the patients of *Sr Martin* and of *Freda and Tacchi* were carried out by cystoscopy, a method which can hardly be considered fully reliable unless there is a complete obstruction. Moreover, the comparability of the material, is reduced by the different intervals between operations and postoperative examination. The series by *Dahle et al.* and *Solomons et al.* were most comparable to the present material (Table II). In the series by *Dahle et al.* and in the present material, the late follow-up examination cannot be taken to have traced conclusively any transient ureteric lesions there may have been.

## STRESS INCONTINENCE

### A Follow-up Study of Operative Treatment

BY

J. SOLEM SKJERAASEN

#### *Introduction*

The term stress incontinence is used for the common condition in women, in which there is involuntary loss of urine on straining, coughing, sneezing or laughing. Kegel (1948) has claimed good results for his method of pelvic floor exercises, but the general opinion is still that the treatment must be operative. Most gynaecologists consider that the first operation for stress incontinence should be a simple vaginal plastic procedure, mostly a modification of the method described by Kelly (1913). For subsequent operations different methods are recommended. Opinions vary as to the failure rate of vaginal repair operations.

At the Department of Obstetrics and Gynaecology, University of Oslo, a large number of patients with stress incontinence are operated on each year. The patients are referred from every part of Norway, and among these several have already had operations in their local hospitals. A follow-up study has been carried out with particular attention to the results of different types of operation, the results of subsequent operations and the time taken for the symptom to recur.

#### *Material and Methods*

In a 10-year period (1955 to 1964) 725 patients with stress incontinence were operated on at the Department of Obstetrics and Gynaecology, University of Oslo. Questionnaires were sent to these 725 women in 1966 and information was obtained from 627. From this number 72 cases had to be excluded. Forty of the women were dead, two had severe senile dementia, and 30

ficient to justify routine postoperative diagnostic measures will remain for each individual gynaecologic surgeon to determine. Routine follow-up examination was omitted as long as the only reliable routine examination was intravenous pyelography. Not until the introduction of isotope renography has reliable post operative follow up examination been possible in practice.

## SUMMARY

An unselected series of 280 patients treated for benign gynaecological conditions by total hysterectomy is presented. The condition of their ureters was examined postoperatively by isotope renography. No instance of a permanent, latent ureteric obstruction was found in the series.

## REFERENCES

- Bratenberg, H. Feichtinger H. and Höfer R. *Geburtsh. u. Frauenheilk* 25 701 1963
- Dahle T. Toverud S. and Trautberg, K. *Acta obst. et gynec. scand* 41 181 1962
- Denneberg, T. *Acta chir. scand.* 118 231 1960
- Felne U. and Bauer A. M., *Fortschr. Röntgenstrahlen* 94 623 1961
- Freda V. C. and Tacchi D. A. *Amer. J. Obstet. Gynec.* 83 406 1962
- Gerble A. B. and Flanagan C. L. *Ibidem* 84 1638 1962
- Higgins C. C. *J.A.M.A.* 199 82, 1967
- Koplowitz J. M. Mitchell J. F. and Bladh W. H. *J.A.M.A.* 193 1032, 1965
- Lawrence J. R. Dolg, A. Knight I. C. S. and Tothill P. *Brit. med. J.* 1 504 1963
- Morrison J. A. *Obst. Gyn. Brit. Cwlth.* 67 66 1960
- Solomon E. Levi E. J. Bauman J. and Baron J. *Surg. Gyn. and Obst.* 111 41 1960
- St. Martin E. C. Trichel B. E. Campbell J. H. and Locke C. U. *J. Urol* 70 51 1953
- Winter C. C. *Radionuclide Renography* Williams and Wilkins Co. Baltimore, 1963
- Zum Winkel A. and Scheer A. E. *Chirurg* 31 487 1960
- Zum Winkel A. Scheer A. E. and Becker J. *Medizinische H* 58, 1960
- Zum Winkel K. *Nierendiagnostik mit Radiosotopen* Georg Thieme Verlag, Stuttgart 1964



Three-hundred-and-fifty of the women had suffered from stress incontinence exclusively while 93 had combined stress and urge incontinence and 112 had stress incontinence with other urinary symptoms such as frequency nocturia, dysuria or incontinence in a recumbent position. Patients with urge incontinence alone were treated conservatively.

Ninety-eight cases out of the 555 women in the follow-up study had a history of one or more previous vaginal operations for stress incontinence. Seventy-nine had been operated on once eighteen twice and one three times before. Twenty of our own patients had subsequent operations in our department in the 10 year period and in these cases only the last operation has been considered.

The preoperative findings on pelvic examination are shown in Table II. Cystocele was the most common finding in the patients

Table II. The Findings at the Preoperative Pelvic Examination before I First Operation II Second Operation III Third Operation

Findings		No. of cases
Cystocele	I	261
	II	17
	III	3
Prolapse of the uterus	I	96
	II	12
	III	2
Descent of the urethra	I	64
	II	13
	III	1
Vaginal scarring	I	37
	II	12
	III	1
Other pathological condition (see text)	I	10
Normal findings on pelvic examination	I	26
	I	457
	II	79
	III	18
Total		555

One woman had been operated upon three times before

patients who according to their records had operations for both stress incontinence and genital prolapse denied any trouble with stress incontinence preoperatively. Thus the follow-up study includes 555 patients.

The diagnosis of stress incontinence in this series was based on the history. Cystoscopy and cystometry were performed routinely. Lateral urethrocystograms were obtained in 24 patients only and showed loss of the posterior urethro-vesical angle in all but one.

Table I shows the age distribution in the follow-up study. Patients between 40 and 59 years constituted the largest group. Only four per cent were older than 70 and 13 per cent less than 40 years. The youngest patient was 23 and the oldest 81 years of age.

It is generally accepted that the stress incontinence is related to pregnancy and damage to the genital tract at the time of delivery. Congenital forms are rare. Among the 555 women in this study only 9 were nulliparous. The largest group - 192 patients - had given birth to two children. Many of them may have developed stress incontinence at the time of their first delivery. It is reasonable to assume that the records do not give complete information about obstetrical complications. Out of the 555 cases 88 had been delivered by forceps, three cases had a complicated delivery with total rupture of the perineal body and four had been delivered by Caesarean section. Thirty patients had children weighing more than 4500 grams at birth. One hundred and fifty five women stated that they developed stress incontinence immediately after a delivery.

Table I The Age Distribution of the 555 Patients in the follow-up Study

Age in Years	No. of Cases
<30	10 (1.8%)
30-39	64 (11.7%)
40-49	181 (32.6%)
50-59	179 (32.2%)
60-69	100 (18.0%)
>70	21 (3.7%)
Total	555

In 45 the incontinence was unchanged and five reported aggravation of their symptom after the operation.

Table III. Results of Different Types of Operation for Stress Incontinence (I. First Operation, II. Second Operation, III. Third Operation)

Operation	No. of Cases			
	Total		Continent Improved	
Modified Kelly	I	70	17	28
			64.3 %	
	II	31	8	5
Modified Kelly colpoperineorrhaphy	III	2	1	
	I	139	51	46
			69.8 %	
Modified Kelly amp of the cervix, colpoperineorrhaphy	II	9	4	2
	III	1	1	
	I	178	87	58
Modified Kelly amp of the cervix			80.3 %	
	II	11	6	5
	III	2	2	
Modified Kelly amp of the cervix	I	5	3	
	II	1		1
	III	1	1	
The interposition operation	I	54	29	11
			74.1 %	
	II	18	5	5
Marshall-Marchetti-Krantz	III	7	4	
	I	2	2	
	II	3	1	1
Other	III	4	2	1
	I	9	3	4
	II	6	4	1
	III	1	1	
	1			
		555	232	166

One woman had her fourth operation for stress incontinence with no improvement

amp amputation

having their first operation. Prolapse of the uterus was less frequently combined with stress incontinence. Descent of the urethra during straining was found in 64 cases. The group named "other pathological conditions" comprises patients with retroversion of the uterus, small fibromyomas and rectocele. In 26 patients there was no obvious abnormality on pelvic examination. Vaginal scarring was more common than recurrence of cystocele and prolapse of the uterus in the patients with a history of previous operations.

Nearly all patients had vaginal operations. During the 10-year period a modified Kelly operation was usually preferred. The suburethral dissection was often combined with urethrolisis. With reefing mattress sutures in the urethro-vesical fascia the surgeons have tried to support the bladder neck. Different materials were used for these "Kelly sutures". Non-absorbable material (silk or nylon) was used in 159 patients and in the others catgut was used. A survey of the different types of operation is given in Table III.

The same type of operation was usually employed for subsequent operations. The Marshall-Marchetti-Krantz operation was performed in only nine cases, and the group "other operations" includes two Aldridge slings and two Ingelman Sundberg operations.

### *Results*

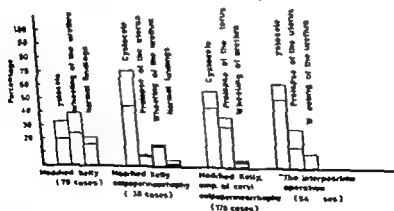
Table IV shows the results of primary and secondary operations postoperatively and at the time of the follow-up study which was done in 1966.

The expression "postoperatively" refers to the time when the patients started a normal life at home. According to the records more than 95 per cent of the patients were continent when they left the hospital, but this information is of little value. It is our experience that patients suffering from stress incontinence often have no leakage of the urine when they live quietly in hospital. Two-hundred-and-ninety-nine women were completely continent postoperatively and 87 were improved in the group of primary operations. The success rate is therefore 84.3 per cent. Twenty-one patients gave no information about the postoperative result,

Table V The Results of the Operations Compared with the Findings on the Preoperative Pelvic Examination in I Primary Operations II. Subsequent Operations

Findings		No. of Cases		
		Total	Continent	Improved
Cystocele	I	261	116	69
			70.9 %	
Protrusion of the uterus	II	20	8	7
	I	96	44	30
			78 %	
Descent of the urethra	II	14	9	3
	I	64	25	27
			81.2 %	
Vaginal scarring	II	14	5	1
	II	50	18	10
			56 %	
Other pathological findings (see text)	I	10	1	6
Normal findings	I	26	6	13
			73.1 %	
	I	457	192	145
	II	98	40	21

Table VI The Late Results of the Most Common Types of Primary Operation in the Series Related to the Findings on the Preoperative Pelvic Examination The Grey Part Represents Continent and Improved Patients



The follow-up study revealed in this group continence in 192 patients and improvement in 145. That means a success rate of 73.7 per cent. In 99 cases the stress incontinence was unchanged and in 10 cases aggravated after the operation. Eleven patients had second operations before this follow-up study was performed.

In Table IV are also seen the results of the subsequent operations. Postoperatively 76.5 per cent were continent or improved compared with 62.2 per cent at the follow up study.

The final results of the different types of operation are shown in Table III and Table V shows the late results related to the preoperative findings on pelvic examination. In Table VI the results are shown in relation both to the type of operation and the preoperative findings.

The success rate of operations among the 350 patients who had stress incontinence only was 74.9 per cent. Among the remaining 205 cases with stress incontinence combined with urgency or other urinary symptoms 66.5 per cent were continent or improved after surgical treatment.

Table IV *The Condition Postoperatively and at the Follow-up Study in I 457 Cases of Primary Operation and II 98 Cases of Subsequent Operations*

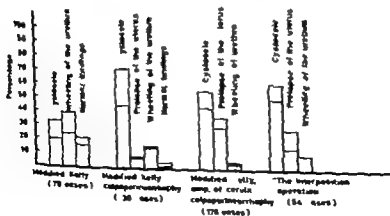
	Postoperatively		Un- changed	Ag- gravated	Un- known
	Con- tinent	Im- proved			
Group I	299	87	45	5	21
	84.3 "			15.5 "	
Group II	60	15	14	4	5
	76.5 "			23.3 "	

	At the Follow-up Study		Un- changed	Ag- gravated	Further Operation
	Con- tinent	Im- proved			
Group I	192	145	99	10	11
	73.7 "			26.3 "	
Group II	40	21	24	10	3
	62.2 "			37.8 "	

Table V The Results of the Operations Compared with the Findings on the Preoperative Pelvic Examination in I Primary Operations II Subsequent Operations

Findings		No. of Cases		
		Total	Continent	Improved
Cystocele	I	281	116	119
			70.9 %	
Prolapse of the uterus	II	20	8	7
	I	96	44	30
			78 %	
Descent of the urethra	II	14	9	3
	I	64	25	27
			81.2 %	
Vaginal scarring	II	14	5	1
	I	50	18	10
			56 %	
Other pathological findings (see text)	I	10	1	6
Normal findings	I	26	6	13
			73.1 %	
	I	457	192	145
	II	98	40	21

Table VI The Late Results of the Most Common Types of Primary Operations in the Series Related to the Findings on the Preoperative Pelvic Examination. The Grey Part Represents Continent and Improved Patients



The success rate among the patients where non absorbable material was used for the Kelly stitches was 65.4 per cent compared with 75.2 per cent in the remaining group.

At the time of the follow-up study 163 patients had recurrent stress incontinence although 79 of these still reported some improvement as a result of their operation. Of the other 84 patients with recurrence 48 reported that they had been completely continent for a while and 36 had shown temporary improvement.

Table VII shows the time interval before the recurrences occurred. The questionnaires from 25 patients gave no information about when their symptoms recurred, and 11 stated that stress incontinence re-appeared gradually after the operation. Of the 79 patients who were considered continent postoperatively and

Table VII. A Survey of Patients with Recurrence of Stress Incontinence and the Time Interval before Recurrence Occurred

Condition Post operatively	Condition at the Follow-up Study Compared with the Pre-operative	No. of Cases				Average Time before recurrence for the Patients in Group III (Years)
		Total	Group I	Group II	Group III	
Completely continent Completely continent	Still improved	79	15	6	58	3.3
	Unchanged aggravated or had further operation	48	1	2	45	2.2
Improved	Unchanged aggravated or had further operation	36	9	3	24	1.6
		163	25	11	127	

Group I. patients with unknown length of time before recurrence

Group II. patients with gradual appearance of recurrence

Group III. patients with a known length of time before recurrence



who were still improved at the follow-up study 17 women were continent for more than five years, four got leakage of the urine during the first six months after the operation, and the remainder

37 patients - were continent from one to five years post-operatively. The average time before the symptom recurred was 3.3 years in this group.

The next group comprises patients with complete continence postoperatively but whose symptom had since completely recurred. The average time before recurrence was shorter in this group - 2.2 years. Eight of the patients were continent for more than five years after the operation, eight got stress incontinence during the first half year after the operation and the remaining 29 had recurrent symptoms from one to five years after the operation. The patients reporting an improvement but not complete cure have the shortest average time before recurrence, 1.6 years. Only one of them was still improved after five years. Nine had a recurrence during the first six months and 14 from one to five years after the operation.

The age distribution among the women with recurrence of stress incontinence seems to be almost the same as in the whole group of patients. There appears to be no higher frequency of recurrences in the menopausal age group. Fourteen patients have had a further operation since the follow up and six became continent or were improved.

There were no postoperative deaths and few serious complications (Table VIII). Ninety-one patients had retention of urine for more than one week after the operation. The cure rate among those with temporary retention was only very slightly better than in the group as a whole. In most cases the indwelling catheter was removed on the fourth day postoperatively. Poor healing of the anterior colporrhaphy was reported in 91 patients. Five patients developed thrombophlebitis and one of these cases was complicated by pulmonary embolism. Urinary fistulae occurred in two patients and both were cured surgically. One woman had a strangulation of the ileum after a Marshall-Marchetti-Krantz operation combined with laparotomy.

Table VIII. *A Survey of Patients with Postoperative Complications and the Results at the Follow-up Study in These Cases*

Complication	No. of cases		
	Total	Continent	Improved
Retention of urine	91	44	25
		75.8 %	
Poor healing of the anterior colporrhaphy	91	31	26
		62.6 %	
Haemorrhage	21	9	6
Urinary infection	26	10	10
Urethro-vaginal fistula	2	2	
Intestinal strangulation	1	1	
Thrombophlebitis	5	2	1
Other	17	11	6
	254	110	74
		72.4 %	

### Discussion

It is difficult to draw firm conclusions from this follow-up study. Only 555 out of the 725 patients who had operations for stress incontinence are included in the study. Like most series the material is not homogeneous. At the preoperative pelvic examination different conditions were found and the patients had different types of operation and the members of the staff did not always use the same technique. The diagnosis of stress incontinence was based mainly on the patient's history and the results had to be evaluated on the subjective impression of the patients. This involves a degree of uncertainty particularly in women stating a postoperative improvement. The conclusions therefore have to be guarded.

The cure rate which usually refers to continent and improved patients varies in the published reports. Millin (1948) and Read (1950) reported late results a little above 70 per cent cured. In a follow-up study Nilsen (1958) found a cure rate of 74 per cent among 110 patients operated on during the years 1950-1954.

Björro and Meylander (1958) found 91.3 per cent of 127 women continent or improved after operation for stress incontinence when a modified Kelly operation with non-absorbable suture material was used. Jeffcoate (1961) reported a 60 per cent cure rate for anterior colporrhaphy on the basis of a two years follow-up study. In Te Lindsa (1962) hands 90 per cent were continent and five per cent improved postoperatively but he did not present the late results. Many gynaecologists claim to have successful results immediately postoperatively in about 90 per cent, however they are aware of the reduced number of cured patients with the elapse of time.

The postoperative results in the present series are poorer than in the above reports, but the longterm results do not differ much. The cure rate in second or subsequent operations is lower than in the primary operations. A number of patients, however have been cured by repeat-operations, even by a third operation. Probably there is a greater risk of stress incontinence being aggravated by a secondary operation. A few of the women who had repeat-operations reported an almost continuous leakage of urine afterwards.

Considering the results in relation to the different types of operation it is obvious both in primary and in secondary operations that the Kelly method has failed in many cases. Where the Kelly operation was combined with a colpoperineorrhaphy and particularly also with an amputation of the cervix, the results were better. This agrees with Bailey (1954) who was "holding a firm belief that practically every case of stress incontinence could be cured by an adequately performed Manchester colporrhaphy. The results of the utero-vesico-vaginal interposition operation are rather good. Our experience with other methods is minimal. The outcome by the Marshall-Marchetti-Krantz operation is remarkable. Five out of seven women having repeat-operations of this type were continent or improved at the time of the follow-up study. In our series of cases the Aldridge sling operation was used once for a third and once for a fourth operation, resulting in complete continence and no improvement, respectively. Ingelman-Sundberg's operation was carried out for two repeat-operations with improvement in one of them. The use of

non-absorbable material for the Kelly sutures" can not be shown to have improved the final results in this series.

In the primary operations a higher cure rate was found in patients with descent of urethra and prolapse of the uterus. The numbers of women in the different groups coming to repeat operations are small. Those with vaginal scarring, though a small number showed a tendency to inferior results.

The choice of operation is certainly connected with the preoperative findings on pelvic examination. The figures in Table VI do not show any clear relation between the final results, the type of operation and the preoperative pelvic findings. There are failures in nearly every group. Patients with prolapse of the uterus have mainly required treatment by the most favourable operations and that may to some extent explain the higher cure rate in this group.

The results seem to be most favourable in the patients who had pure stress incontinence without other urinary symptoms. In the other groups there are probably some patients included who were not suffering from true stress incontinence. Operative treatment may have been unwisely chosen in these cases.

The increased recurrence rate as time passes is confirmed. Of the patients with complete continence postoperatively only a few had recurrent symptoms during the first six months, but the deterioration in the results even after five years is remarkable. Patients who were only improved by the operation tend to have an earlier return to the preoperative degree of stress incontinence.

It has been suggested that recurrences may be due to inadequately performed operations (Read 1950). Our study supports this view as a few particularly skilled surgeons achieved better results than those in training who in our series had the poorest results, though as a rule they were assisted by senior gynaecologists.

There were few serious complications. It has been assumed that a successful operation causes retention of urine for one or two weeks after the operation. The results in the 91 women who had retention of urine for more than one week after the operation are not convincingly superior to those for all patients. It has

to be mentioned that some questionnaires indicate continuous micturition trouble with urinary infections. Probably some of these patients still have a reduced ability to empty the bladder completely. In our experience poor healing of the anterior colporrhaphy can lower the cure rate.

The results of this follow-up study show the inadequacy of our surgical treatment of stress incontinence, as one fourth of the patients have no lasting improvement from the operation. Better results might possibly be obtained by more careful selection of patients with more refined diagnostic techniques including routine X-ray examination of the posterior urethro-vesical angle and simultaneous recording of the intra-urethral and intra-vesical pressures (Enkhörning, 1961). In this series the therapy has been selected only by considering the patients history and the findings on the preoperative pelvic examination. Green (1962) claimed to have improved his results by separating the patients in two groups: those with loss of the posterior urethro-vesical angle in one group and in the other group those with rotational descent of the urethra in addition to loss of the angle. In the latter group he had performed a more extensive operation. Patients with great shortening of the urethra may be selected for an anterior urethropexy (Lapides 1960).

Nevertheless, the fact that some skilled operators have very good final results, should encourage every gynaecologist to improve his surgical technique.

### SUMMARY

A follow-up study of the results of surgical treatment has been undertaken among 555 patients who had operations for stress incontinence at the Department of Obstetrics and Gynaecology, University of Oslo, during the years 1955-1964. Nearly all had vaginal operations. A modified Kelly operation was usually preferred, often combined with an amputation of the cervix and a colpoperineorrhaphy. The operation was the primary treatment in 457 cases but 98 cases had a second or subsequent operation. Postoperatively 84.3 per cent were continent or improved after the primary operations compared with 76.5 per cent following

non-absorbable material for "the Kelly sutures" can not be shown to have improved the final results in this series.

In the primary operations a higher cure rate was found in patients with descent of urethra and prolapse of the uterus. The numbers of women in the different groups coming to repeat operations are small. Those with vaginal scarring, though a small number showed a tendency to inferior results.

The choice of operation is certainly connected with the pre-operative findings on pelvic examination. The figures in Table VI do not show any clear relation between the final results, the type of operation and the preoperative pelvic findings. There are failures in nearly every group. Patients with prolapse of the uterus have mainly required treatment by the most favourable operations and that may to some extent explain the higher cure rate in this group.

The results seem to be most favourable in the patients who had pure stress incontinence without other urinary symptoms. In the other groups there are probably some patients included who were not suffering from true stress incontinence. Operative treatment may have been unwisely chosen in these cases.

The increased recurrence rate as time passes is confirmed. Of the patients with complete continence postoperatively only a few had recurrent symptoms during the first six months, but the deterioration in the results even after five years is remarkable. Patients who were only improved by the operation tend to have an earlier return to the preoperative degree of stress incontinence.

It has been suggested that recurrences may be due to inadequately performed operations (Read 1950). Our study supports this view as a few particularly skilled surgeons achieved better results than those in training who in our series had the poorest results, though as a rule they were assisted by senior gynaecologists.

There were few serious complications. It has been assumed that a successful operation causes retention of urine for one or two weeks after the operation. The results in the 91 women who had retention of urine for more than one week after the operation are not convincingly superior to those for all patients. It has

to be mentioned that some questionnaires indicate continuous micturition trouble with urinary infections. Probably some of these patients still have a reduced ability to empty the bladder completely. In our experience poor healing of the anterior colporrhaphy can lower the cure rate.

The results of this follow-up study show the inadequacy of our surgical treatment of stress incontinence, as one fourth of the patients have no lasting improvement from the operation. Better results might possibly be obtained by more careful selection of patients with more refined diagnostic techniques including routine X-ray examination of the posterior urethro-vesical angle and simultaneous recording of the intra-urethral and intra-vesical pressures (Enkström, 1961). In this series the therapy has been selected only by considering the patients history and the findings on the preoperative pelvic examination. Green (1962) claimed to have improved his results by separating the patients in two groups, those with loss of the posterior urethro-vesical angle in one group and in the other group those with rotational descent of the urethra in addition to loss of the angle. In the latter group he had performed a more extensive operation. Patients with great shortening of the urethra may be selected for an anterior urethropexy (Lapides 1960).

Nevertheless, the fact that some skilled operators have very good final results, should encourage every gynaecologist to improve his surgical technique.

### SUMMARY

A follow-up study of the results of surgical treatment has been undertaken among 553 patients who had operations for stress incontinence at the Department of Obstetrics and Gynaecology University of Oslo, during the years 1955-1964. Nearly all had vaginal operations. A modified Kelly operation was usually preferred, often combined with an amputation of the cervix and a colpoperineorrhaphy. The operation was the primary treatment in 457 cases but 98 cases had a second or subsequent operation. Postoperatively 84.3 per cent were continent or improved after the primary operations compared with 76.5 per cent following

non absorbable material for the Kelly sutures can not be shown to have improved the final results in this series.

In the primary operations a higher cure rate was found in patients with descent of urethra and prolapse of the uterus. The numbers of women in the different groups coming to repeat operations are small. Those with vaginal scarring, though a small number showed a tendency to inferior results.

The choice of operation is certainly connected with the pre-operative findings on pelvic examination. The figures in Table VI do not show any clear relation between the final results the type of operation and the preoperative pelvic findings. There are failures in nearly every group. Patients with prolapse of the uterus have mainly required treatment by the most favourable operations and that may to some extent explain the higher cure rate in this group.

The results seem to be most favourable in the patients who had pure stress incontinence without other urinary symptoms. In the other groups there are probably some patients included who were not suffering from true stress incontinence. Operative treatment may have been unwisely chosen in these cases.

The increased recurrence rate as time passes is confirmed. Of the patients with complete continence postoperatively only a few had recurrent symptoms during the first six months, but the deterioration in the results even after five years is remarkable. Patients who were only improved by the operation tend to have an earlier return to the preoperative degree of stress incontinence.

It has been suggested that recurrences may be due to inadequately performed operations (Road 1950). Our study supports this view as a few particularly skilled surgeons achieved better results than those in training who in our series had the poorest results, though as a rule they were assisted by senior gynaecologists.

There were few serious complications. It has been assumed that a successful operation causes retention of urine for one or two weeks after the operation. The results in the 91 women who had retention of urine for more than one week after the operation are not convincingly superior to those for all patients. It has



## CANCER OF THE FEMALE URETHRA

A Clinical Study of 25 Cases

BY

EGIL SKJERAASEN

Malignant tumours of the female urethra are uncommon. Zinzer (1955) found that the rate of urethral cancer related to the incidence of cancer in the female genital organs was 0.83 per cent. During the years 1953-65 altogether 28 new cases of urethral cancer in women were reported to the Cancer Registry of Norway. In the same period 4449 cases of invasive cancer of the cervix were detected in the country. The incidence of malignant urethral tumours thus constituted only 0.63 per cent of that of cervical malignancies. This explains why the reported series of patients with urethral cancer have been so small. The largest series published up to 1966 consisted of 79 cases (Grabstald *et al.* 1966).

A variety of treatments have been tried in cancer of the urethra in females. Radium irradiation, external irradiation, or combined radiotherapy and surgery have resulted in 5-year survival rates in the range of 30-43 per cent (Fricke *et al.* 1949, Ruch *et al.* 1952, Monaco *et al.* 1958). Sallinen *et al.* (1965) reported on 29 patients treated mainly by electrocoagulation and local radium, and with a 5-year survival of 38 per cent. Treatment principally with local radium has been advocated by several workers, resulting in 5-year survival rates of from 28 to 67 per cent (Göbel *et al.* 1947, Zinzer 1955, Staibitz *et al.* 1955, Hultberg 1956, Wasserburger 1956).

Against the background of the small series of cases and the relatively poor results of treatment hitherto reported, it was of

repeat-operations. At later follow-up the cure rate was 73.7 and 62.2 per cent respectively.

Many patients with recurrent symptoms had been completely continent for several years, some for more than five years.

There were no postoperative deaths and few serious complications.

#### REFERENCES

- Bailey K. V. *J. Obst. & Gynaec. Brit. Emp.* 61: 291, 1954.  
Björö A. and Meflænder O. *Tidskr. Norske Lægeforen.*, 78: 414, 1958.  
Enhördning, G., *Acta chir. scand. Suppl.* 276, 1961.  
Green T. H. *Am. J. Obst. & Gynec.* 83: 632, 1962.  
Jeffcoate T. N. A. *J. Roy. Coll. Surg. Edinburgh* 7: 29, 1961.  
Kegel A. H. *Am. J. Obst. & Gynec.* 56: 238, 1948.  
Kelly H. A. *Urol. & Cutan. Rev.* 17: 291, 1913.  
Lapides J., Ajemian E. P., Stewart B. H., Lichwardt J. R. and Breakley B. A. *Surg. Gynec. Obstet.* 111: 224, 1960.  
Mullin T. and Read, C. D. *Post. Grad. med. J.* 24: 3, 1948.  
Read C. D. *Proc. Roy. Soc. Med.* 43: 255, 1950.  
Nilsen P. A. *Nord. Med.* 59: 182, 1958.  
Te Linde R. W. *Operative Gynaecology* Lippincott Philadelphia, 1962 (s. 183).

Received on June 19, 1968

Table II.

Symptoms	No. of Cases
Bleeding	16
Dysuria or frequency	14
Urinary retention	7
Urinary incontinence	2
Tumour in the urethral orifice	2
Discharge	3
Dyspareunia	3

was recorded in 22 cases. One patient had noted spotting of blood for many years. In the other 21 patients symptoms had been present from one month to two years, and on average 8.7 months.

A clinical diagnosis of urethral caruncle had been made in three patients 2-5 years before a biopsy from the lesion disclosed cancer.

Cytological smears had been taken from the urethral orifice in only two patients. In both cases malignant cells were found.

The tumour was clearly limited to the anterior part of urethra in eight cases (Table III). Vulvo-urethral forms with extension to the adjoining parts of vulva or the outer part of the anterior vaginal wall were seen in four cases. Tumour involving the posterior part of urethra or with extension to the surroundings was found in 13 cases.

Table III Follow-up Results Related to Location of Tumours

Location	Total No. of Cases	Alive without Residual Disease	Dead from Intercurrent Disease	Dead from Cancer
Anterior part of urethra	8	2	2	4
Vulvo-urethral	4	1	1	2
Posterior part of urethra	13	2		11
Total	25	5	3	17

interest to review our own series of urethral cancer in the female dating back to the opening of the hospital in 1932.

### *Material*

Between the years 1932 and 1964 29 women with tumours classified as cancer of the urethra were admitted to the Norwegian Radium Hospital. In four cases the histological examination did not confirm the clinical diagnosis and these were therefore excluded. Accordingly the series comprises 25 cases of cancer of the urethra confirmed microscopically.

The age distribution of these cases is shown in Table I. The youngest patient was 42 years and the oldest 73 years of age at the time the diagnosis was established. The majority of the women (19 patients) were in the age group 50-69 years.

Table I. Age Distribution in 25 Women with Cancer of Urethra

Age Groups	No. of Cases
40-49 years	2
50-59 years	11
60-69 years	8
70-79 years	4
Total	25

Average age: 60.7 years

There were five nulliparous patients three patients had given birth to one child, and six to two children, while seven patients had had three or more deliveries. In four cases the parity was not stated in the records.

### *Clinical findings*

The symptoms are given in Table II. Haematuria or spotting of blood with or without painful or frequent micturition were the most common complaints being observed in 24 of the 25 patients. In one case dyspareunia due to narrowing of the vaginal introitus was the only symptom. The duration of the symptoms

2400 r to 5000 r. During the period 1952-60 orthovoltage X-ray treatment was given to eight patients. The skin doses in these cases were from 3000 r to 3600 r. Since 1961 a 31 MeV betatron machine has been used in seven patients, with a calculated tumour dose of between 4000 r and 6000 r.

### *Follow-up*

The follow-up in 1967 of the 25 patients showed that five patients were alive and without recurrence after an observation time from 3 to 6 years. Two patients died of intercurrent disease after 10 years and 17 years of observation respectively. The recurrence free group thus consisted of seven cases, one of which was treated by resection of the urethra, one was treated by radium and external betatron irradiation, while the remaining five were subjected to a combination of surgery and radiotherapy. Four of the seven recurrence free patients were found in the group of tumours located in the anterior part of urethra (Table III). Only one of the five patients with lymph node metastases was found to be without recurrence.

One patient died from pulmonary embolism two weeks after excision of the urethra. Seventeen patients are considered to have died of urethral cancer. The average time of survival after the initial treatment was 23 months.

### *Discussion*

The reported series from the Norwegian Radium Hospital confirms that urethral cancer in women is an uncommon disease with a grave prognosis.

The poor over-all results reported in the literature are the more impressive as observations indicate that urethral cancers are apt to remain localized for a considerable period of time (Gahrstål *et al.* 1966). Crucial points might be that the disease is uncommon and that the symptoms are not alarming and may often be neglected. The small published series indicate that even specialists in gynaecological cancer generally obtain only a limited personal experience of female urethral cancer. The varied

### *Pathology*

The majority of the lesions were classified as squamous cell carcinomas (15 cases). Transitional cell carcinoma was found in five cases, adenocarcinoma in three cases and undifferentiated malignant tumour in three cases. One of the patients with a squamous cell lesion had been treated four years previously for cancer of the uterine cervix stage I. The examination revealed a papillomatous squamous cell carcinoma, and a metastasis from the cervical cancer could not be excluded. Since no other signs of recurrence were found however the case was included in the urethral cancer series.

Enlarged inguinal lymph nodes were noted in 13 patients. Groin dissection with histological examination of the nodes was performed in six of these and in five cases tumour metastases were found.

### *Therapy*

Treatment consisted of surgery, radiotherapy or a combination of both. One cachectic patient with a very advanced cancer had no treatment. Two patients were treated exclusively by surgery, eight by radiation alone and fourteen patients were subjected to combined treatment.

Partial resection of urethra was performed in four patients, in two of which diathermy was used. Total excision of the urethra was carried out in seven patients. In five of these some type of urinary diversion was established and plastic reconstruction of urethra was performed in the other two cases.

Radiotherapy consisted of local treatment with radium or external irradiation, separately or in combination. Different types of radium source have been applied both in the urethra directly into the tumour (radium needles) or in the vagina. It is impossible retrospectively to calculate tumour doses, the amount of radium varying between 750 and 3600 mg hours.

The methods of external irradiation varied considerably in the period under discussion. Telerradium therapy was used in seven patients during the years 1932-1958. The beam was directed to the vulva and the inguinal areas, skin doses ranging from

2400 r to 5000 r. During the period 1952-60 orthovoltage X-ray treatment was given to eight patients. The skin doses in these cases were from 3000 r to 3600 r. Since 1961 a 31 MeV betatron machine has been used in seven patients, with a calculated tumour dose of between 4000 r and 6000 r.

### *Follow-up*

The follow-up in 1967 of the 25 patients showed that five patients were alive and without recurrence after an observation time from 3 to 6 years. Two patients died of intercurrent disease after 10 years and 17 years of observation respectively. The recurrence free group thus consisted of seven cases, one of which was treated by resection of the urethra, one was treated by radium and external betatron irradiation, while the remaining five were subjected to a combination of surgery and radiotherapy. Four of the seven recurrence free patients were found in the group of tumours located in the anterior part of urethra (Table III). Only one of the five patients with lymph node metastases was found to be without recurrence.

One patient died from pulmonary embolism two weeks after excision of the urethra. Seventeen patients are considered to have died of urethral cancer. The average time of survival after the initial treatment was 23 months.

### *Discussion*

The reported series from the Norwegian Radium Hospital confirms that urethral cancer in women is an uncommon disease with a grave prognosis.

The poor over-all results reported in the literature are the more impressive as observations indicate that urethral cancers are apt to remain localized for a considerable period of time (Grabstald *et al.* 1966). Crucial points might be that the disease is uncommon, and that the symptoms are not alarming and may often be neglected. The small published series indicate that even specialists in gynaecological cancer generally obtain only a limited personal experience of female urethral cancer. The varied

The majority of the lesions were classified as squamous cell carcinomas (15 cases). Transitional cell carcinoma was found in five cases, adenocarcinoma in three cases and undifferentiated malignant tumour in three cases. One of the patients with a squamous cell lesion had been treated four years previously for cancer of the uterine cervix stage I. The examination revealed a papillomatous squamous cell carcinoma, and a metastasis from the cervical cancer could not be excluded. Since no other signs of recurrence were found, however, the case was included in the urethral cancer series.

Enlarged inguinal lymph nodes were noted in 13 patients. Groin dissection with histological examination of the nodes was performed in six of these and in five cases tumour metastases were found.

### Therapy

Treatment consisted of surgery, radiotherapy or a combination of both. One cachectic patient with a very advanced cancer had no treatment. Two patients were treated exclusively by surgery, eight by radiation alone and fourteen patients were subjected to combined treatment.

Partial resection of urethra was performed in four patients, in two of which diathermy was used. Total excision of the urethra was carried out in seven patients. In five of these some type of urinary diversion was established, and plastic reconstruction of urethra was performed in the other two cases.

Radiotherapy consisted of local treatment with radium or external irradiation, separately or in combination. Different types of radium source have been applied both in the urethra directly into the tumour (radium needles) or in the vagina. It is impossible retrospectively to calculate tumour doses, the amount of radium varying between 750 and 3600 mg hours.

The methods of external irradiation varied considerably in the period under discussion. Telerradium therapy was used in seven patients during the years 1932-1958. The beam was directed to the vulva and the inguinal areas, skin doses ranging from



ical removal of the urethra and external 31 MeV betatron radiation.

It is noteworthy that four of the eight patients with tumours confined to the urethral orifice showed no evidence of recurrence, while only two of 13 patients with tumours in the posterior part of urethra were free from recurrence. *Wasserburger* (1956) found one 5-year survival among six patients with tumours in the posterior urethra whereas 22 of 24 patients with growths in the anterior part of urethra were alive after 5 years. These findings show that the location of the tumour has a great bearing upon the prognosis.

During the last decades local radium treatment in urethral cancer in women has become popular. It is likely that the mode of radium application is of the utmost importance. Most authors seem to prefer radium needles (*Göbel et al.* 1947 *Ruch et al.* 1952 *Straubitz et al.* 1955 *Wasserburger* 1956 *Monaco et al.* 1958) *Grabstald et al.* (1966) have also used interstitial radium treatment according to the technique described by *Henschke et al.* (1963). On the other hand extensive surgery including urethrocystectomy or anterior exenteration, has been undertaken in posterior urethral tumours, and the results have been encouraging (*Straubitz et al.* 1955 *Grabstald et al.* 1966).

Metastases to the inguinal lymph nodes were observed in five out of 25 patients. This corresponds well with other authors reporting metastases to the groins in 20-25 per cent of cases (*Göbel et al.* 1947 *Wasserburger* 1956 *Sallinen et al.* 1965) *Grabstald et al.* (1966) examined the pelvic lymph nodes in 26 of 79 patients and metastases were revealed in 13. Inguinal metastases have been treated by X-ray irradiation (*Fricke et al.* 1949 *Sallinen et al.* 1965) groin dissection (*Monaco et al.* 1958) or irradiation and surgery (*Hultberg*, 1956).

In order to obtain a more reliable basis for the handling of urethral cancer it seems necessary to have larger series with more standardized treatment. The treatment should be centralized in special institutions for cancer therapy. Cooperation between such centres might contribute to a better understanding of this form of cancer.

and often unsystematic therapeutic methods used in these cases seem to support such a statement.

It is a common finding that patients with urethral cancer have micturition troubles (Monaco *et al.* 1958 Marshall *et al.* 1960 Grabstald *et al.* 1966). In the present series all patients except one complained of dysuria, frequency or bleeding. It follows that urethral cancer should be considered in adult women with urinary symptoms.

Most tumours are located in the outer part of the urethra (Fricke *et al.* 1949 Wasserburger 1956 Monaco *et al.* 1958 Sallinen *et al.* 1965). They are easily seen and palpated, and are readily accessible for biopsy. The most common type of tumour is a squamous cell carcinoma (McCrea 1952) and this suggests that exfoliative cytology can be of value in the diagnosis of urethral cancer. In two patients in the present series a cytological smear was taken and malignant cells were found in both. Colposcopy is another method that may be of importance for the early diagnosis of this disease. Koller (1966) reported on 42 patients with vulval lesions including three cases of urethral carcinoma and stated that the colpophotographic findings were much of the same type as those found in carcinoma of the cervix.

There has been discussion whether caruncle of the urethra may be related to urethral cancer. Carcinomatous changes inside caruncles have been demonstrated (Ratner *et al.* 1948 Marshall *et al.* 1960). Hence the possibility of malignant development in these lesions can hardly be excluded. Malignant tumours on the other hand can be erroneously diagnosed as caruncles. Marshall *et al.* (1960) found six cases of carcinoma and three cases of Bowen's disease in 376 cases clinically interpreted as caruncles. Accordingly it has been claimed that all urethral growths should be subjected to histological examination (Menville 1935 Walther 1943 Marshall *et al.* 1960).

Treatment in the present series of 25 cases was most often surgery combined with radiotherapy. The limited number of cases and the large number of different combinations of treatment preclude definite statements as to the effectiveness of the various therapeutic measures. Improved preliminary results, however, have been observed since 1964 in a few patients treated by rad

ical removal of the urethra and external 31 MeV betatron radiation.

It is noteworthy that four of the eight patients with tumours confined to the urethral orifice showed no evidence of recurrence, while only two of 13 patients with tumours in the posterior part of urethra were free from recurrence. *Wasserburger* (1956) found one 5-year survival among six patients with tumours in the posterior urethra whereas 22 of 24 patients with growths in the anterior part of urethra were alive after 5 years. These findings show that the location of the tumour has a great bearing upon the prognosis.

During the last decades local radium treatment in urethral cancer in women has become popular. It is likely that the mode of radium application is of the utmost importance. Most authors seem to prefer radium needles (*Göbel et al.* 1947 *Ruch et al.* 1952 *Staubitz et al.* 1955 *Wasserburger* 1956 *Monaco et al.* 1958) *Grabstald et al.* (1966) have also used interstitial radium treatment according to the technique described by *Henschke et al.* (1953). On the other hand extensive surgery including urethrocystectomy or anterior exenteration, has been undertaken in posterior urethral tumours, and the results have been encouraging (*Staubitz et al.* 1955 *Grabstald et al.* 1966).

Metastases to the inguinal lymph nodes were observed in five out of 25 patients. This corresponds well with other authors reporting metastases to the groins in 20–25 per cent of cases (*Göbel et al.* 1947 *Wasserburger* 1956 *Sallinen et al.* 1965). *Grabstald et al.* (1966) examined the pelvic lymph nodes in 26 of 79 patients and metastases were revealed in 13. Inguinal metastases have been treated by X-ray irradiation (*Fricke et al.* 1949 *Sallinen et al.* 1965) groin dissection (*Monaco et al.* 1958) or irradiation and surgery (*Hultberg*, 1956).

In order to obtain a more reliable basis for the handling of urethral cancer it seems necessary to have larger series with more standardized treatment. The treatment should be centralized in special institutions for cancer therapy. Cooperation between such centres might contribute to a better understanding of this form of cancer.

## SUMMARY

Between 1932 and 1964 25 women with urethral cancer were seen in the Norwegian Radium Hospital. The majority of the patients were treated by a combination of radiotherapy and surgery.

Five patients were free from recurrence after an observation period of 3 to 6 years, and two other patients died of intercurrent disease after several years of observation. Seventeen patients died of spread of the urethral cancer and one of post operative pulmonary embolism.

The series shows that urethral cancer should be borne in mind in patients with micturition symptoms. Cytological smears are likely to be useful as a screening test in suspicious cases, and colposcopy might be of diagnostic value.

The results of treatment are more clearly related to the site of the tumour than to the actual therapy employed. In growths limited to the anterior urethra well planned irradiation as well as surgery may cure the lesions whereas the poor outcome in tumours located in the posterior urethra indicates that more intensive irradiation or extensive surgical intervention is necessary.

A centralized treatment of patients with urethral tumours in special institutions for cancer therapy is recommended.

## REFERENCES

- Fricke R. E. and McMillan J. T. *Radiology* 53 533 1949  
 Grubstald H. Hilaris B. Henschke U. and Whitmore W. F. *J. Amer. med. Ass.* 197 835 1966  
 Göbel A. and Vonesen A. *Strahlentherapie* 70 529 1947  
 Henschke U. H. Hilaris B. S. and Mahan G. D. *Amer. J. Roentgenol.* 90 386 1963  
 Hultberg, S. *Strahlentherapie* 99 11 1956  
 Koller O. *Acta obstet. gynec. scand.* 45 E8 1966  
 Marshall F. C. Uson A. C. and Melcose M. M. *Surg. Gynec. Obstet.* 110 723 1960  
 McCrea L. E. *Urol. Surv.* 2 85 1952  
 Menzies J. G. *Surg. Gynec. Obstet.* 61 229 193  
 Monaco A. P. Murphy G. B. and Dowling W. *Cancer (Philad.)* 11 1219 1958  
 Ratner M. and Schneiderman C. *Canad. med. Ass. J.* 58 373, 1948

- Ruck, R. M., Frericks J. B. and Aronson A. N. *Cancer* (Philad.) 5 748, 1952
- Salzman, A. and Koudounis, M. *Ann. Chir. Gynaec. Penn.* 54 237 1965
- Steinbeiz W. J. Carden L. M. Oberhirscher O. J. Leuz M. H. and Murphy W. T. *J. Urol.* (Baltimore) 73 1045, 1955
- Walther H. W. E., *J. Urol.* (Baltimore) 50 380 1943
- Waserburger K., *Strahlentherapie* 101 485 1956
- Zinner H. K. in *Seitz Amtesch. Biol. u. Pathol. des Weibes* vol. 10 Urban & Schwarzenberg, Berlin-Wien 1955, III 660

Received on June 19 1968



## Cancer of the Female Urethra

- Rack, R. M., Frerichs, J. B., and Aronson, A. N. *Cancer (Phila.)* 5: 44, 1952
- Seitman, A., and Komisarides M. *Ann. Chir. Gynaec. Fern.* 54: 237, 1963
- Sinabatz, W. J., Carden, L. M., Oberkircher, O. J., Lent, M. H., and Murphy, W. T. *J. Urol. (Baltimore)* 73: 1045, 1955
- Welcher, H. W. E., *J. Urol. (Baltimore)* 50: 380, 1943
- Waserberger, K. *Strahlentherapie* 101: 483, 1950
- Zinner, H.-K., in *Sertiz Aszuresch. Biol. u. Pathol. des Weibes* vol. 10 Urban & Schwarzenberg, Berlin-Wien 1953, p. 660

Received on June 19, 1968

## SUMMARY

Between 1932 and 1964 25 women with urethral cancer were seen in the Norwegian Radium Hospital. The majority of the patients were treated by a combination of radiotherapy and surgery.

Five patients were free from recurrence after an observation period of 3 to 6 years and two other patients died of intercurrent disease after several years of observation. Seventeen patients died of spread of the urethral cancer and one of post operative pulmonary embolism.

The series shows that urethral cancer should be borne in mind in patients with micturition symptoms. Cytological smears are likely to be useful as a screening test in suspicious cases and colposcopy might be of diagnostic value.

The results of treatment are more clearly related to the site of the tumour than to the actual therapy employed. In growths limited to the anterior urethra well planned irradiation as well as surgery may cure the lesions whereas the poor outcome in tumours located in the posterior urethra indicates that more intensive irradiation or extensive surgical intervention is necessary.

A centralized treatment of patients with urethral tumours in special institutions for cancer therapy is recommended.

## REFERENCES

- Fricke R. E. and McMillan J. T. *Radiology* 57 533, 1949  
 Gabstald H., Hilariis B., Henschke U. and Whitmore W. F. *J. Amer. med. Ass.* 197 835, 1960  
 Göbel A. and Vonesse A. *Strahlentherapie* 76 529, 1947  
 Henschke U. K., Hilariis B. S. and Mahan G. D. *Amer. J. Roentgenol.* 90 350, 1963  
 Hultberg, S. *Strahlentherapie* 99 171, 1946  
 Koller O. *Acta obstet. gynec. scand.* 45 89, 1966  
 Marshall F. C., Uson A. C. and Mellor M. M. *Surg. Gynec. Obstet.* 110 723, 1960  
 McCrea L. E. *Urol. Surv.* 2 85, 1952  
 Menville J. G. *Surg. Gynec. Obstet.* 61 229, 193  
 Monaco A. P., Murphy G. B. and Dowling W. *Cancer (Philad.)* 11 1, 1953  
 Ratner M. and Schneiderman C. *Canad. med. Ass. J.* 59 373, 1949



- Rack, R. M. Frerichs J. B. and Arneson A. N. *Cancer* (Philad.) 5 748, 1952
- Sellmes, A., and Koulondes M., *Ann. Chir. Gynaec. Fenn.* 54 237 1965
- Staubitz W. J. Carden, L. M. Oberkircher O. J. Lent M. H. and Murphy W. T. J. *Urol. (Baltimore)* 73 1045, 1955
- Waliker H. W. E. J. *Urol. (Baltimore)* 50 380 1943
- Weserberger K., *Strahlentherapie* 101 485, 1956
- Zheuer H. K. in *Seitz-Anzeich. Biol. u. Pathol. des Weibes* vol. 10 Urban & Schwarzenberg, Berlin-Wien 1955, p. 660

Received on June 19 1968